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STUDIES ON THE LEGUME ROOT NODULE BACTERIA

II. THE PRODUCTION AND BEHAVIOR OF COLONIAL MUTANTS PRODUCED BY X-RAY IRRADIATION[1,*]

BY D. C. JORDAN[2]

Abstract

Four distinct types of colonial mutation were obtained by X-ray irradiation of a parasitic strain of alfalfa *Rhizobium*. Two types were procured from an effective strain. Colonial morphology remained stable throughout serial transfer on artificial medium, but changed considerably after one plant passage. Three variants derived from the parasitic parent were found to be efficient in nitrogen fixation when tested by sterile plant growth procedures and the Virtanen technique. This efficiency increased during the course of two plant passages. Colonial variation and effectivity changes occurred independently of one another.

Introduction

Since the first report of bacterial colony variation by Firtsch (4) in 1888 an immense volume of literature has accumulated on this important phase of bacteriology. Studies in this field have a tendency to be directed toward the animal pathogens, primarily because of their importance in disease production. Although such mutants have been investigated in a number of other bacterial species reports dealing with rhizobia have been very meager.

Almon and Baldwin (2) found six culture types differing from typical *Rhizobium trifolii* colonies with regard to color and gum production. They were obtained by a variety of treatments including filtration, and subsequent cultivation of the filtrates, growth in certain media, storage in sterilized soil, and by bacteriophage action.

The first work on rough colonies of the nodule bacteria was begun by Israilsky and Starygin (7) in 1930 and was continued by Israilsky and Leonowitsch (6).

Nutman (12) perceived that stock cultures of both an effective and an ineffective strain of *Rhizobium* showed an occasional tendency to produce new colony types. These particular variants were convex and often umbilicate or lobed on the surface. They were also very butyrous and the surface was smooth, but not as smooth as that of "normal" colonies. Kleczkowska (9), working with bacteriophage-resistant mutants of *R. trifolii*, found rough, intermediate, and smooth forms. Although the colonial morphology was retained

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intact up to a period of three years on artificial media, most of the strains reverted to the parent type upon a single plant passage. In certain cases the new characters were retained after this passage. It was postulated that the plant exerted a strong selective action on the invading organisms. Colonial mutation and nitrogen fixing ability occurred independently of one another and mutants differing from the parent in nitrogen fixing power were either stable or unstable in this respect.

There is apparently no published report in which the so-called "mutagenic" treatments have been used on rhizobia. Such treatments are known to produce gene changes in organisms such as *Drosophila*, but their action toward bacteria is debatable owing to the paucity of information concerning the nuclear apparatus of these microorganisms. Most of the methods used with the nodule bacteria have been selective procedures whereby the parental population is inhibited or destroyed and certain random mutations allowed to reproduce freely.

The present work is devoted to studies on artificially induced colonial variants of alfalfa root nodule bacteria, in order to perfect a method efficient in the production of such mutants and to determine whether a relationship exists between colony types and nitrogen fixing efficiency, a feature which Kleczkowska did not find with *R. trifolii*.

In this particular study the term "rough" will refer only to colonial appearance and not to any specific serological behavior.

Methods

Among the various mutagens used were X rays, ultraviolet light, uranium nitrate, and diazomethane. The X-ray method was found to be the more productive of variants and only this procedure will be given.

For this work a parasitic *Rhizobium* strain (R_{20}) and an effective *Rhizobium* strain (R_{21}) were used. Both these strains were of the alfalfa-sweet clover cross-inoculation group and their effectiveness had been previously determined by the Virtanen technique (8).

A small loopful of growth from a 96 hr. slant culture of the test organism was suspended in 5 ml. of 0.85% saline solution and brought up to a total volume of 15 ml. with the same diluent. A 4 ml. aliquot of this final dilution was placed in a small sterile Petri plate (4.5 cm. in diameter), the top of which was replaced by a sheet of cellophane. This plate was placed 3 cm. from the target of the X-ray tube of a therapy-type machine and the bacterial suspension subjected to a dosage of 45,000 r. units of unfiltered X rays. This involved running the unit for 32 min., 12 sec., at a 90 kv. peak and at 5 ma., the machine having been previously calibrated in air with a Victoreen dosimeter. Backscatter was avoided by placing a lead-rubber pad beneath the Petri plate. Viable cells were usually reduced from approximately 1 billion per ml. to 55,000 per ml. by this irradiation process.

Various dilutions of the treated suspension were plated out on Lochhead's medium (11) and the plates examined for colonial mutants after incubation

at 25°C. for 7-14 days. In some cases an aliquot of the same suspension was examined by the Davis–Lederberg penicillin method for the concentration of biochemical mutants (3,10) and then colonies were selected. The mutants found were subjected to single cell isolation and success was attained in a large number of cases.

All the isolates were tested for purity by cultural and fermentation reactions, and their ability to fix molecular nitrogen was determined by means of sterile plant growth assemblies and the Virtanen technique (8). The host plant was Grimm alfalfa.

Results

Four types of colonial mutation were found after X-ray treatment of R_{20} and two after irradiation of R_{21}. The colonial morphology was maintained with no apparent change throughout a large number of serial transfers on artificial medium. Descriptions of these mutant types are given in the table, together with the code numbers of some representative isolations.

The "rough" colonies could not be distinguished from the smooth types by the Pampana test (13), the crystal violet method that White and Wilson (14) used for *Brucella*, or by means of 2,3,5-triphenyltetrazolium chloride (5).

The greenhouse efficiency tests demonstrated that three of the colonial mutants of the parasite R_{20} were now efficient in the fixation of free nitrogen.

TABLE I

DESCRIPTION OF COLONIAL VARIANT TYPES OBTAINED BY THE USE OF X RAYS

Colonial description	Representative isolations	Parent strain*
Smooth Umbonate Mucoid	$R_{20\text{-}16}$ $R_{20\text{-}17}$*	R_{20}
Smooth Umbilicate Mucoid	$R_{20\text{-}19}$* $R_{20\text{-}20}$	R_{20}
Slightly "rough" Convex Mucoid	$R_{20\text{-}27}$* $R_{20\text{-}28}$	R_{20}
Very "rough" Umbilicate Waxy	$R_{20\text{-}31}$*	R_{20}
"Rough" plateau with smooth sides Entire colony removed with needle	$R_{21\text{-}26}$	R_{21}
Slightly "rough" Umbilicate Butyrous	$R_{21\text{-}27}$*	R_{21}

**Single cell isolations.*

These variants, $R_{20\text{-}27}$, $R_{20\text{-}28}$, and $R_{20\text{-}31}$ were all "rough" and produced leghemoglobin values, respectively, of 115.59, 112.53, and 148.88 gamma per gram of nodule tissue. All the other variants tested behaved as their parent strains, those derived from R_{20} being parasitic and those derived from R_{21} being effective.

A considerable degree of colonial change was found among some of the mutants after re-isolation from the nodules, testing for purity, and plating out on Lochhead's medium. $R_{20\text{-}27}$ colonies were now umbilicate, $R_{20\text{-}28}$ and $R_{20\text{-}31}$ had reverted from the "rough" to the smooth type, $R_{21\text{-}27}$ colonies became smooth and convex, and $R_{21\text{-}26}$ became considerably less butyrous in consistency.

After this primary plant passage the three effective R_{20} variants were again tested on greenhouse-grown alfalfa plants. They maintained their effectiveness and in all cases gave leghemoglobin values greater than that previously observed. Thus it appeared that these cultures had actually become more efficient in the fixation process.

Discussion

Although many microbiologists have reported decreases in the effectiveness of rhizobia by means of certain treatments, there are, to the author's knowledge only two published reports dealing with an increase in this effectiveness. The increases were brought about by plant passage (1) and by bacteriophage activity (9), both of which apparently act through a process of selection rather than by the creation of nuclear changes. The present investigation is a report of an increase in *Rhizobium* effectiveness occurring through the agency of artificially induced mutation, brought about principally by the use of ionizing radiation.

After a single plant passage the newly acquired colonial characteristics of the various mutants changed. This agrees somewhat with Kleczkowska who maintains that this results from a strong selective action, present in the nodule tissue, which is directed against colonial variants of rhizobia. In her work, however, the variants reverted to the parent type while in the present work the reversion was not always completely to the parental state and, in some cases, only minor changes occurred. Thus, this postulated selective activity may not always be powerful enough to effect complete reversion.

Since the effective R_{20} variants maintained their effectivity after a second colonial change it is concluded that effectivity and colonial variation occur independently of one another, in agreement with the work on *R. trifolii* (9).

Since ineffective mutants have been said to be developed more easily from effective strains than vice versa it has been concluded (9) that bacteriophage action, when present, may increase the proportion of ineffective bacteria and thus indirectly affect plant growth. The study herein described shows, however, that effective strains may be developed from parasitic strains by artificial methods, a condition which, in addition to random mutation, could occur in the soil. These latter factors may function as stabilizing agents, offsetting the deleterious effects of 'phage action by the production of effective mutants

which would be, perhaps, "immune" to lytic activity. The processes taking place are, in all probability, not as simple as this and a dynamic state involving large efficiency shifts may occur within the soil. Bacteriophage may select ineffective strains so that the entire *Rhizobium* population within a given soil becomes essentially ineffective. This may account for the prevalence of such strains in certain areas. This condition might continue indefinitely, but effective mutants are still capable of being produced and any change in "selection stress" might favor their development to the detriment of the ineffective types. In this manner legume growth would be affected adversely or beneficially depending upon which efficiency type had the ascendancy at the time of nodulation.

The soil factors influencing *Rhizobium* mutation may be few or many, and in all likelihood include both selection agents and true mutagens of unknown identity.

The use of X rays may also be an important laboratory tool for the production of root nodule bacteria superior to their parent strains in nitrogen fixing ability. If such derived strains are stable or can be made so then commercial legume inoculants could be made much more effective than they are at present.

Mutational work among the nodule bacteria may be the means of solving many of the problems confronting the students of symbiotic nitrogen fixation. Such mutations could well represent the forms of these bacteria as they occur in the "wild" state. Laboratory cultures, although subconsciously referred to as the norm, may in reality be variations derived from the "wild" types by spontaneous or induced mutation (coupled with selection) or temporary adaptation. Thus mutational processes provide a ready means for the conversion of rhizobia into the various states of discontinuous variation capable of existence within the soil.

Preliminary work in our laboratory has revealed that irradiation is also capable of producing auxtrophic *Rhizobium* mutants which can be isolated by various techniques. These biochemically deficient organisms merit further study because they can be used to advantage in nutritional studies and perhaps in the further elucidation of the fixation mechanism itself.

Acknowledgments

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THE DECOMPOSITION OF DITHIOCARBAMATE FUNGICIDES, WITH SPECIAL REFERENCE TO THE VOLATILE PRODUCTS[1]

By L. E. Lopatecki and W. Newton[2]

Abstract

The gaseous exchange accompanying decomposition of dithiocarbamate fungicides was measured in the Warburg manometer. Sodium diethyl dithiocarbamate decomposed under slightly acid conditions, producing carbon disulphide and a salt of diethylamine. The insoluble zinc and iron salts of dimethyl dithiocarbamic acid also decomposed in a similar fashion, but no carbon disulphide was given off from the more stable copper salt of this compound. The rate of decomposition of these metallic salts fell in the following decreasing order:

$$\overset{+}{\text{Na}} > \overset{++}{\text{Zn}} > \overset{+++}{\text{Fe}} > \overset{++}{\text{Cu}}\,.$$

Disodium ethylene *bis*-dithiocarbamate (nabam) decomposed under slightly acid conditions to produce approximately equal volumes of hydrogen sulphide and carbon disulphide, and presumably left a residue of ethylene thiourea. In distilled water, on the other hand, nabam underwent a slow basic hydrolysis and oxidation, with absorption of approximately two volumes of oxygen, and evolution of one volume of carbon disulphide. Apparently the sulphur fraction which is evolved as hydrogen sulphide from nabam under acid conditions is, under basic conditions, oxidized in solution by atmospheric oxygen to sulphate.

Introduction

Parker-Rhodes (3) first suggested that a volatile toxicant, carbon disulphide, is involved in the fungicidal action of the dithiocarbamates. This toxicant was considered to be a product of the action of acidic spore secretions upon the dithiocarbamate, the over-all reaction being as follows:

$$\text{S}=\text{C}\begin{matrix}\diagup\text{NR}_2\\ \diagdown\text{SNa}\end{matrix} + \text{H}_2\text{O} \rightleftharpoons \text{NHR}_2 + \text{CS}_2 + \text{NaOH},$$

the amine and alkali being neutralized by the acid present. The dialkylamine formed by this reaction was considered also to contribute to the fungicidal action of the dithiocarbamate. Later Barratt and Horsfall (1) pointed out the relatively low fungicidal value of carbon disulphide and the amines. They suggested instead that the action of nabam and related dithiocarbamates was due to the more toxic hydrogen sulphide, coupled with the ability of these compounds to form insoluble salts with trace metals essential in mold metabolism. Recently, Rich and Horsfall (5) found that a gaseous toxicant is evolved from nabam solutions in addition to hydrogen sulphide. They suggest that identification of this toxicant may assist in accounting for the fungicidal properties of nabam.

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In the investigations reported here, a Warburg manometer was used to follow the decomposition of dithiocarbamate fungicides in acid and aqueous solution, and to identify the volatile decomposition products.

Materials and Methods

The dithiocarbamates used in these experiments were obtained from the following sources:
Sodium diethyl dithiocarbamate—Eastman Kodak Company.
The cupric, ferric (ferbam),* and zinc (ziram)* salts of dimethyl dithiocarbamic acid by courtesy of Canadian Industries Limited, and disodium ethylene *bis*-dithiocarbamate (nabam),* by courtesy of Dr. J. Horsfall, Connecticut Agricultural Experiment Station.

Measurements of the gases evolved from dithiocarbamates were made in a conventional Warburg manometer. Except in specified cases, the following manometer technique was adopted: 1 ml. of $3\times10^{-3}M$ dithiocarbamate was added to a flask side arm, 0.4 ml. of Sorensen's $M/15$ phosphate buffer placed in the main flask, and water added to the flask to make a final fluid volume of 3.0 ml. Following an equilibration period of 10 min. in the water bath at 28°C., the dithiocarbamate in the side arm was tipped into the buffer in the main flask, and manometer readings recorded at regular intervals.

Two gas absorbents were used in the flask center wells, a 10% aqueous solution of potassium hydroxide, and a 30% solution of this base in absolute ethyl alcohol. Prior to the addition of 0.2 ml. of absorbent to the center well, the well rim was wax-coated to prevent creeping of absorbent solution. A small wad of glass wool was then placed in the well to increase the absorptive surface. The customary procedure of using filter paper for the latter purpose introduced an error, since alcoholic potash in contact with the paper absorbed atmospheric oxygen. Control flasks containing alcoholic potash and 3.0 ml. of water were utilized to make corrections for the small vapor pressure registered by the alcoholic solutions.

Results

Sodium Diethyl Dithiocarbamate

The influence of pH upon the decomposition of the soluble compound, sodium diethyl dithiocarbamate, was determined in the Warburg manometer. The rate of carbon disulphide evolution from this substance in the presence of buffers of pH ranging from 5.0 to 7.0 is shown in Fig. 1.

Fig. 1 shows that sodium diethyl dithiocarbamate is relatively stable at pH 7.0, but commences to decompose with evolution of a gas as soon as the reaction becomes acid. It was confirmed that the gas evolved was carbon disulphide by its action on aqueous and alcoholic potash, and by the performance of Reith's test (4). Thus the gas was not absorbed by aqueous potash, but was absorbed rapidly and completely by alcoholic potash. Reith's test was performed by first absorbing the gas in 0.2 ml. of 5% alcoholic

*Common name adopted by the American Phytopathological Society.

potash, and then adding one drop of 1/50 *N* copper acetate, followed by 0.2 ml. of 4.0 *N* acetic acid. The presence of carbon disulphide was confirmed by the formation of yellow copper ethyl xanthate.

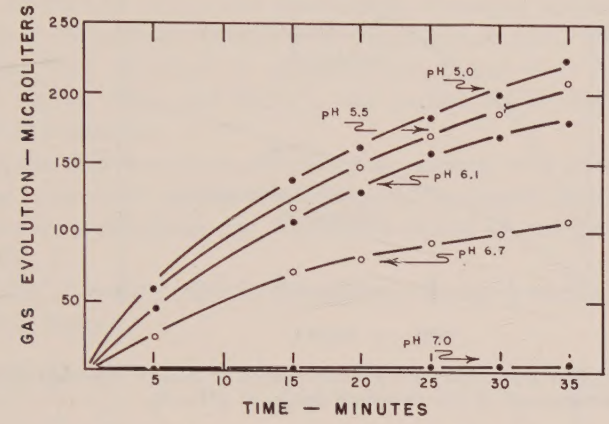


FIG. 1. Influence of pH of *M*/15 phosphate buffer upon carbon disulphide evolution from sodium diethyl dithiocarbamate.

Confirmation was also obtained that an amine was a second decomposition product of this dithiocarbamate at a slightly acid reaction. An amine salt was isolated following decomposition of sodium diethyl dithiocarbamate at pH 5.0 with hydrochloric acid. This salt had a m.p. of 218.2°C., which agrees closely with the m.p. of 219-220°C. recorded for diethylamine hydrochloride.

Metal Salts of Dimethyl Dithiocarbamic Acid

Measurements were then made of carbon disulphide evolution from the insoluble copper salt of dimethyl dithiocarbamic acid, and from the insoluble fungicides ferbam and ziram. Decomposition of these compounds proved to be slow compared to that of the soluble sodium salt. In order, therefore, to obtain larger manometer measurements, 1.0 ml. of $3\times10^{-2}M$ dithiocarbamate suspension and 2.0 ml. of pH 5.0 buffer were added to each flask. Under these conditions, no carbon disulphide could be detected from the relatively stable copper salt. The rates of evolution of carbon disulphide from the other two compounds are shown in Fig. 2.

The gas evolved by both metal salts was identified by previously described tests as carbon disulphide, while a secondary decomposition product in the form of an amine was indicated by a rise in pH which accompanied decomposition of these two compounds. Apparently therefore, in the presence of dilute acid, decomposition of insoluble metal complexes of dimethyl dithiocarbamic acid proceeds in a similar fashion to that of the soluble sodium salt. The rate of decomposition, however, appears to fall in the following decreasing order:

$$\overset{+}{\text{Na}} > \overset{++}{\text{Zn}} > \overset{+++}{\text{Fe}} > \overset{++}{\text{Cu}}.$$

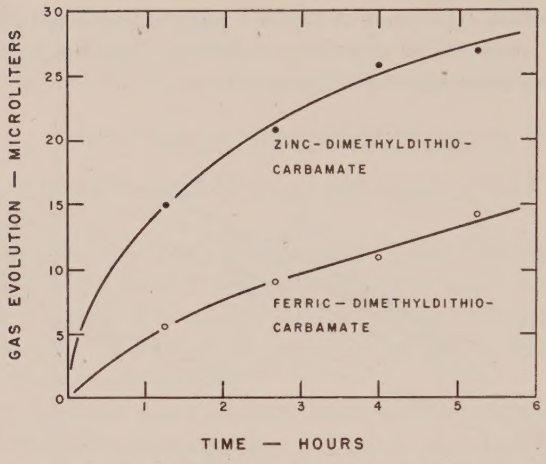


FIG. 2. Carbon disulphide evolution from insoluble metallic salts of dimethyl dithiocarbamic acid in the presence of $M/15$ phosphate buffer of pH 5.0.

Nabam

Acid Hydrolysis

The gases evolved from nabam in the presence of an acid buffer were measured in a similar fashion in the Warburg manometer. For this determination, 1.0 ml. of $3\times10^{-2}M$ nabam was added to a flask side arm, and 2.0 ml. of pH 6.0 buffer placed in the main flask. The differential absorption of the ensuing gases on aqueous and alcoholic potash was determined as before.

Since the gas evolved from nabam was partially absorbed by aqueous, and totally absorbed by alcoholic potash, it was concluded that the gas was a mixture. Tests with lead acetate indicated that the fraction of gas absorbed

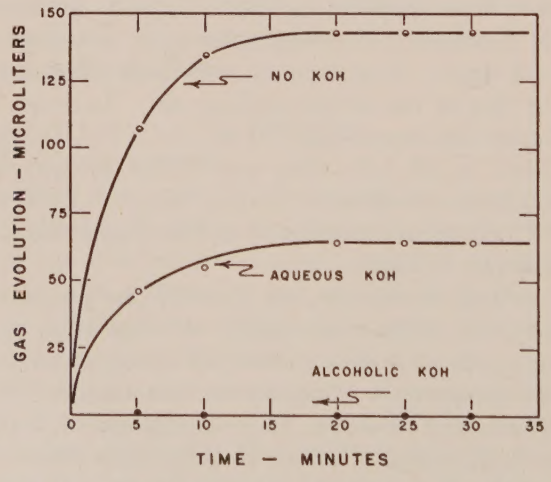
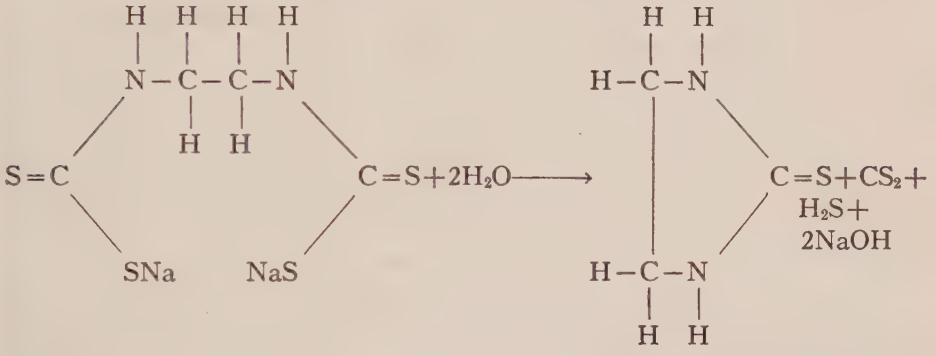


FIG. 3. Volumes of gases evolved from nabam in the presence of $M/15$ phosphate buffer of pH 5.0, and the differential absorption of these gases by aqueous and alcoholic potash. The top curve represents the total volume of carbon disulphide and hydrogen sulphide, while the middle curve represents the volume of carbon disulphide only.

by aqueous potash was hydrogen sulphide. On the other hand, Reith's procedure and lead acetate tests showed that both hydrogen sulphide and carbon disulphide were absorbed by the alcoholic potash. Since Reith's test could not be carried out in the presence of hydrogen sulphide, this gas was first absorbed by aqueous potash in the flash center well. The remaining gas fraction was then absorbed in alcoholic potash run into the flask side arm through a vented plug, and Reith's test performed by adding the reagents directly to this solution.

Utilizing the difference in readings of manometers with and without aqueous potash, the volumes of carbon disulphide and hydrogen sulphide evolved from nabam were then calculated by Warburg's Direct Method. These results are shown in Fig. 3.

Apparently therefore, nabam decomposes in the presence of weak acid in the following fashion:



Nabam Ethylene thiourea

Two additional facts tend to substantiate this mode of decomposition. First, ethylene thiourea has been reported as a breakdown product of nabam by Barratt and Horsfall (1). Secondly, the one-to-one ratio of the volumes of hydrogen sulphide to carbon disulphide evolved in theory, approaches the 1.2-to-one ratio obtained experimentally (see Fig. 3).

Basic Hydrolysis

Evolution of gas from nabam in distilled water was similarly measured in the Warburg manometer. For this purpose, 3.0 ml. portions of a $10^{-2}M$ solution of nabam in distilled water were added to the main flasks, while aqueous and alcoholic potash were used as absorbents in the center wells.

A gradual shift in pH occurred in distilled water solutions of nabam, the pH rising from an initial reading of 7.3 to a final reading of 10.5. Manometer measurements indicated that the gaseous exchange which occurred over these basic solutions involved both gas evolution and oxygen absorption. Since the evolved gas was absorbed by alcoholic, but not by aqueous potash, and produced a positive Reith's reaction, it was concluded that carbon disulphide alone was present. From the difference in readings of manometers with and

without alcoholic potash the oxygen uptake and carbon disulphide evolution were calculated by Warburg's Direct Method. These results are shown graphically in Fig. 4.

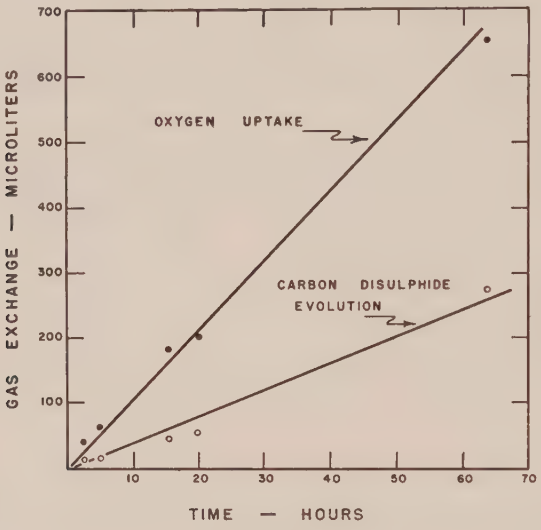


FIG. 4. Rate of oxygen uptake and carbon disulphide evolution from nabam in aqueous solution.

Fig. 4 indicates that, in distilled water solution, oxidation of one or more of the decomposition products of nabam occurs. Among the final products of oxidation, sulphite and thiosulphate proved to be absent, while the presence of sulphate was demonstrated by the barium chloride test.

Apparently nabam undergoes a basic hydrolysis in distilled water, and decomposes with evolution of carbon disulphide, but not of hydrogen sulphide. Presumably under these conditions the sulphide sulphur remains in solution, possibly as sodium sulphide, and is oxidized by atmospheric oxygen to sulphate. The gaseous exchange required by this supposition is as follows:

1. Nabam + $2H_2O$ - - - - - - - - - ethylene thiourea + CS_2 + H_2S + 2NaOH
2. H_2S + 2NaOH - - - - - - - - - - Na_2S + $2H_2O$
3. Na_2S + $2O_2$ - - - - - - - - - - - - Na_2SO_4

According to this breakdown scheme, two volumes of oxygen are required for sulphide oxidation for every one volume of carbon disulphide evolved. Data from Fig. 4, on the other hand, indicate that oxygen uptake exceeds carbon disulphide output by approximately 2.4 times. However, this discrepancy may be explained by the fact that, as seen from Fig. 3, the volume of hydrogen sulphide evolved from nabam, and hence the sulphide sulphur available for oxidation, is 1.2 times above theoretical.

Discussion

Apparently nabam may undergo acid hydrolysis evolving hydrogen sulphide and carbon disulphide, or basic hydrolysis, when carbon disulphide alone is evolved. On the other hand, the tertiary amine compounds, sodium diethyl dithiocarbamate, ferbam, and ziram, undergo acid hydrolysis with evolution of carbon disulphide only. These facts clarify a number of features of gas evolution from the dithiocarbamates, and may help explain some features of their fungicidal action.

That nabam could in fact decompose with evolution of carbon disulphide and hydrogen sulphide was previously considered by Barratt and Horsfall (1), but rejected mainly for two reasons. Firstly, carbon disulphide was not identified, and secondly, this type of breakdown did not appear to account for the low fungicidal values of tertiary amine analogues of nabam. They assumed that if the carbon disulphide–hydrogen sulphide type of breakdown occurred in nabam, then it should also occur with the tertiary amine analogues. They assumed also that this similarity of breakdown would result in similar fungicidal values for nabam and these analogues. However, since other tertiary amine dithiocarbamates upon acid hydrolysis evolve carbon disulphide only, perhaps tertiary amine analogues of nabam do the same. Since carbon disulphide is less toxic than hydrogen sulphide, this may account for the low fungicidal action of these tertiary amine analogues.

An unexplained feature of gas evolution from nabam reported by Rich and Horsfall (5) was that in the presence of *Stemphylium* spores, hydrogen sulphide was given off from dilute solutions, while from concentrated solutions, an unidentified toxicant was evolved. The explanation of this phenomenon seemingly is found in the action of acidic spore secretions upon nabam, since Parker-Rhodes (3) reported *Stemphylium* spore secretions possessed a slightly acid reaction. Apparently these spore secretions produced an acid hydrolysis of dilute nabam solutions, with consequent evolution of hydrogen sulphide and undetected carbon disulphide. On the other hand, since nabam is in the form of a basic sodium salt, the pH of concentrated nabam solutions was not reduced appreciably by the spore secretions. Consequently, these solutions underwent a basic hydrolysis, and evolved carbon disulphide only.

A second feature reported by Rich and Horsfall (5) of the gas evolved from nabam solutions was the unusually flat slope of the dosage–response curves produced by this gas against *Stemphylium* spores. However, they qualified their data by mentioning that these curves possibly were artifacts, resulting from an error in the assumption that the gas concentration over nabam solutions was directly proportional to the concentration of nabam in solution. It is considered that these curves were indeed artifacts, resulting from their 24-hr. test period being insufficient for the complete basic hydrolysis of nabam. Consequently, the potential gas evolution from these solutions was never realized.

Finally, recent reports by Stoddard (6), Haasis and Ellis (2), and Thomas (7) suggest that in some cases nabam and its zinc salt may act as chemotherapeutic agents in controlling plant disease. Owing to the instability of

nabam in the presence of acid, it is unlikely that this substance is translocated unchanged within the plant. Thus if chemotherapeutic action is exhibited by nabam, the action is due presumably to one or more of its breakdown products.

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THE INFLUENCE OF POLYPLOIDY ON THE X-RAY SENSITIVITY OF CELLS[1]

By Charles J. Bishop[2]

Abstract

A study has been made of the comparative X-ray sensitivity of cells of diploid and tetraploid species of *Tradescantia* at two different resting stages during the development of the pollen grains. At the resting stage midway between meiosis and the pollen grain division, no significant difference was found in the number of breaks per chromosome in the two species. At the resting stage just following the pollen grain division, the tetraploid chromosomes showed a slightly higher break frequency, a difference which was statistically significant. This increase was due, at least in part, to the presence of a small portion of cells which showed an abnormally high sensitivity. From a detailed study of the cells at each stage and the types of aberrations induced it is concluded that the chromosomes of each species are of equal sensitivity, and that any difference in the reaction of the diploid and tetraploid cells to irradiation is due simply to the numerical difference in the chromosomes of the cells, or to differences in the nuclear stage when X-rayed.

Introduction

The relationship of polyploidy to X-ray sensitivity is a problem which has already received considerable attention. It is a question of both theoretical and practical importance. From a theoretical point of view, polyploidy is one of the many variables which has an influence on the sensitivity of cells to irradiation, and so offers a possible means of learning more about the fundamental way in which X rays may cause their effects. From a practical point of view, it is an important consideration in the use of radiation therapy in the treatment of cancer, where the malignant growth may include polyploid cells or mixtures of polyploid and diploid cells.

There have been two methods used in the past in attempting to solve this problem. One has been the exposure of seeds or plants of different species in a polyploid series to irradiation, and then the comparison of the survival of the plants in relation to their particular degree of polyploidy. A number of quite extensive experiments of this type (e.g. Stadler (10), Fröier *et al.* (3), Smith (8)) have been carried out, and have shown quite conclusively that, within a particular genus, the polyploid species are more resistant to irradiation than are the diploid species.

In some of the cereal plants an attempt has been made (Fröier *et al.* (4), Smith (7, 8)) to correlate these effects with cytological changes. This has been done by recording either the frequency of anaphase bridges in root-tip divisions, or of reciprocal translocations produced by X-raying pollen and observed in the meiotic divisions of the plants of the next generation. These results have indicated an increase in X-ray induced aberrations in a direct and

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roughly linear proportion to the degree of polyploidy. In none of this material, however, has there been an attempt to analyze completely all the chromosome alterations produced by the irradiation.

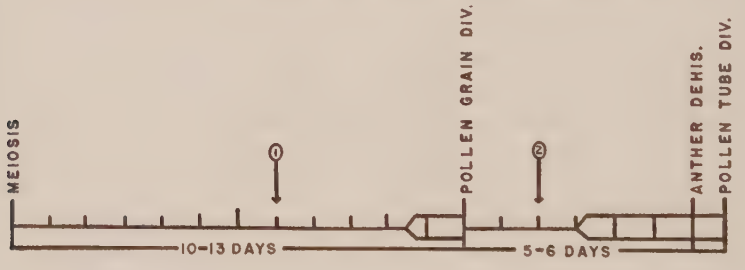
Such a complete analysis has been limited to work on different species of *Tradescantia*. The obvious advantage of a complete analysis is that it gives a more accurate check of the chromosome sensitivity, and makes it possible to determine whether the effects are simply dependent on the numerical difference in the chromosome number, or whether there is a more fundamental change affecting X-ray sensitivity.

Sax and Swanson (6), in a paper on the general subject of the comparative sensitivity of cells to X rays, included a short section on the influence of polyploidy. They found that the tetraploid species of *Tradescantia* had a lower sensitivity than the diploid when the analysis was made on a per-chromosome basis, and indicated the influence of a factor more fundamental than simply a change in the number of chromosomes.

Materials and Methods

Inflorescences of the two species *Tradescantia paludosa* Anders. & Woodson (2*n*) and *Tradescantia virginiana* L. (4*n*) were cut and exposed at the same time to a dosage of either 400 or 240 roentgens of X rays at the rate of 50 r. per minute. The X-ray tube was operated at 140 kv. and 5 ma., and the dosages were recorded by means of a Victoreen dosimeter and automatic timer.

In the first part of this work, permanent acetocarmine smears were prepared at the time of the pollen grain division, five days after irradiation with 400 r. of X rays. This meant that the cells were X-rayed during the resting stage about midway between meiosis and the pollen grain division (Fig. 1). At that



POLLEN GRAIN DEVELOPMENT IN TRADESCANTIA

FIG. 1. Pollen grain development in *Tradescantia*. (The figures 1, 2 indicate the stages X-rayed.)

time all the cells were in a uniform stage, and as all chromosomes were single stranded, only the chromosome type of aberrations were produced.

In the second part of the work, pollen was cultured (Bishop (1)) from flowers opening on the fourth and fifth days after irradiation with 240 r. of

X rays. At the time of X-raying these cells were at the resting stage following the pollen grain division and previous to the doubling of the chromosomes in the generative nucleus, which takes place about three days before another dehiscence (Fig. 1). The results from this material were obtained by an analysis at the metaphase stage of the pollen tube division.

In the analysis of both stages the types of chromosome aberrations scored included (1) simple acentric fragments, (2) dot deletions, (3) dicentric chromosomes, (4) rings, centric, and acentric, (5) tricentric chromosomes, (6) dicentric rings, (7) quadricentric chromosomes, as shown in Fig. 2. Dot deletions were considered to be minute rings and were calculated as involving two breaks in 60% of the cases. This was based on the work of Rick (5) reported in 1940.

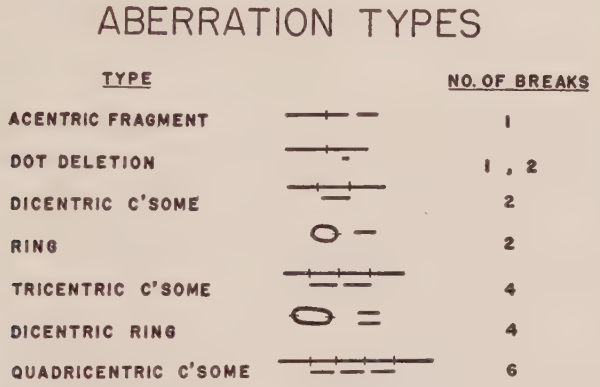


FIG. 2. Types of X-ray induced chromosome aberrations.

Results

With the data obtained from the analysis at the two metaphase stages, it was possible to compare the sensitivity of the diploid and tetraploid cells when in a uniform resting stage. Let us first consider the analysis made at the pollen grain division and representing cells X-rayed during the resting stage about midway between meiosis and the pollen grain division. When a comparison of the diploid and tetraploid material was made on a cellular basis (Table I) it was seen that the tetraploid cells were much more sensitive, there being 5.73 breaks per cell compared with only 2.69 breaks per cell in the diploid.

However, when a comparison of the two types was based on the number of breaks per chromosome, it was found that there was no significant difference between the two species, there being 0.45 breaks per chromosome in the diploid and 0.48 breaks in the tetraploid.

These results would seem to indicate that the chromosomes of the two species have the same sensitivity to X rays, the only difference being that in the tetraploid cells there are twice as many chromosomes, and thus twice the chance that one of those in the cell will be broken by the X rays.

TABLE I

FREQUENCY OF X-RAY INDUCED BREAKS IN DIPLOID AND TETRAPLOID *Tradescantia* CHROMOSOMES

Stage X-rayed	Stage scored	Dose	Number of c'somes		$2n$	$4n$	χ^2	p
Postmeiotic resting stage	Pollen grain division	400 r.	5532	Breaks per cell	2.69	5.73	72.5	<0.01
				Breaks per chromosome	0.45	0.48	1.77	0.19
Post P. G. D. resting stage	Pollen tube division	240 r.	8412	Breaks per cell	2.28	5.49	146.7	<0.01
				Breaks per chromosome	0.38	0.46	21.0	<0.01

A similar comparison was made based on an analysis at the pollen tube division. These cells were X-rayed at the resting stage following the pollen grain division. This resting stage was actually considerably more sensitive than the previous one, as shown by the fact that about the same frequency of aberrations was obtained with slightly more than half the X-ray dosage—240 r. instead of 400 r.

This comparison (Table I) also showed the tetraploid cells to have a much higher frequency of breaks per cell, the figures being 2.28 and 5.49. On a per-chromosome basis the breakage frequency was more nearly alike, but there was still a significant difference, the tetraploid being more sensitive, 0.46 breaks per chromosome compared with 0.38 breaks per chromosome.

In order to study more accurately this variation in the results from the two experiments, graphs were prepared showing the frequency distribution of the different number of breaks per cell. In the data from the postmeiotic resting stage (Fig. 3) the cells show a uniform distribution with the spread of the curves attributable to chance factors, and the difference between them shown by a statistical comparison to be highly significant.

The data from the resting stage following the pollen grain division (Fig. 4) is less uniform. The pollen used for this analysis was cultured on both the fourth and fifth days after irradiation. As will be seen from Fig. 1, this resting stage is of very short duration. Pollen cultured on the sixth day after irradiation develops from cells which were X-rayed at the time of the pollen grain division, and aberrations produced previous to this stage appear in the pollen grains in the form of micronuclei. Pollen cultured on the third day after irradiation or sooner includes only cells in which the chromosomes have become doubled in the initial stages of the prophase of the pollen tube division.

Comparisons were made (Table II) of the sensitivity of the diploid and tetraploid cells on each of the two days, and on both days the tetraploid material was found to be more than twice as sensitive as the diploid. On a per-chromosome basis the difference though smaller was still significant, with

the tetraploid showing the higher breakage frequency. However, there was also a significant difference between the material of the two days for both the diploid and tetraploid cells, with the chromosomes in both cases showing a lower sensitivity when X-rayed on the fifth day before anther dehiscence than on the fourth day.

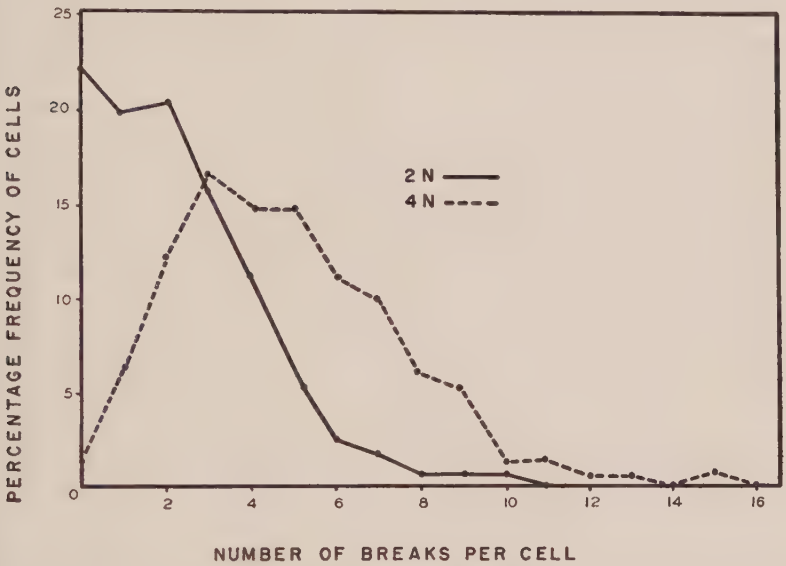


FIG. 3. Frequency distribution of breaks per cell in microspores X-rayed at the postmeiotic resting stage.

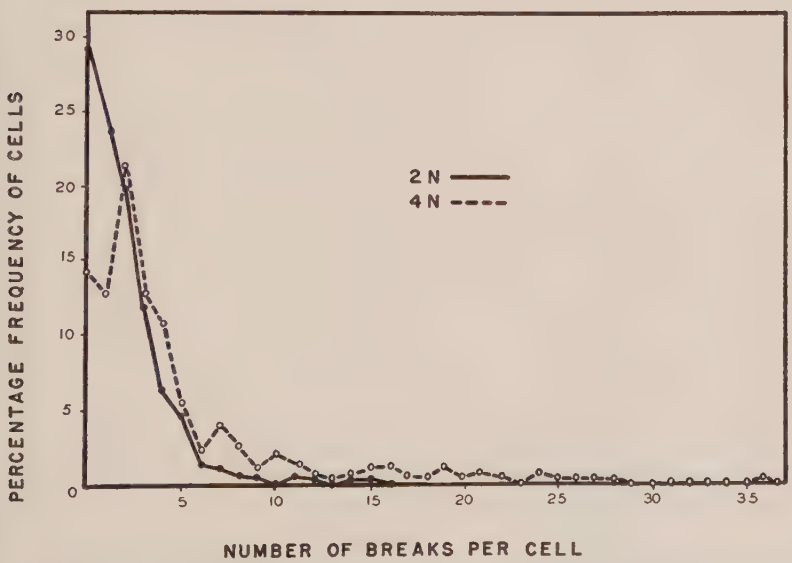


FIG. 4. Frequency distribution of breaks per cell in pollen grains X-rayed at the resting stage following the pollen grain mitosis.

TABLE II

FREQUENCY OF CHROMOSOMAL BREAKS IN THE POLLEN GRAINS OF DIPLOID AND TETRAPLOID *Tradescantia* X-RAYED FOUR AND FIVE DAYS BEFORE ANTHER DEHISCENCE

Species	Day X-rayed*	No. cells	No. c'somes	Dicentrics	Rings	Acentric fragments	Dot** deletions	Total breaks	Breaks per cell	Breaks per c'some
2N	4th	356	2136	53 11.4%	7 1.5%	413 44.7%	253 43.6%	937.8	2.63	0.44
	5th	344	2064	57 17.4%	14 4.2%	84 12.8%	269 65.6%	656.4	1.91	0.32
	Total	700	4200	110 13.8%	21 2.6%	497 31.1%	522 52.4%	1594.2	2.28	0.38
4N	4th	171	2052	72 13.2%	9 1.7%	392 36.0%	333 49.1%	1086.8	6.32	0.53
	5th	180	2160	57 13.4%	16 3.8%	231 27.6%	290 55.2%	841.0	4.67	0.39
	Total	351	4212	129 13.4%	25 2.6%	623 32.3%	623 51.6%	1927.8	5.49	0.46

**Days before anther dehiscence.*

*** 60% of dot deletions were counted as two breaks, 40% as one break.*

Discussion

While the determination of the chromosome X-ray sensitivity at the post-meiotic resting stage seems to indicate that the chromosomes of the diploid and tetraploid cells are of equal sensitivity, the results from the postmitotic resting stage are less definite. The period of the pollen grain mitosis is known to be one of high sensitivity (2). As this occurs about the sixth day before anther dehiscence, and the cells showed a greater sensitivity on the fourth day than on the fifth day, it would seem to indicate that the change in sensitivity was not due to the influence of sensitive stages of the pollen grain division. In the other direction the prophase of the generative nucleus division is initiated, as shown by the effective doubling of the chromosomes on the third day before dehiscence. Nonsynchronization of stages of the cells in the anthers, with some of them entering the more sensitive stage at the time of chromosome doubling (Bishop (2)), could conceivably be the reason for this change in sensitivity.

This is supported by an examination of the frequency distribution of the number of breaks per cell (Fig. 4), where it will be seen that in the tetraploid there were a considerable number of cells (about 10%) which showed an abnormally high breakage frequency, as high as 36 breaks when the mode was two breaks per cell. Because of the somewhat slower nuclear cycle of the tetraploid, the cells of this material would be in a slightly later stage when X-rayed, and a small portion of them may have reached the stage of the

prophase chromosome doubling. In such a case it might be more nearly accurate to compare the fourth day cells of the diploid with the fifth day cells of the tetraploid or some intermediate stage.

In the analysis made at the postmeiotic resting stage, the duration of the nondividing state is much longer, and as there would be no marked change in the sensitivity over a period of several days the nuclear stages of the cells were well synchronized and the deviation in sensitivity did not occur. Because of the error introduced by the variability of the nuclear stage of the cells, this postmeiotic resting stage is probably more reliable as a true indicator of the relative sensitivity of diploid and tetraploid chromosomes. However, in the postmitotic resting stage there was sufficient difference to indicate that on a per-cell basis the tetraploid cells were much more sensitive than the diploid, and that on a per-chromosome basis they were more nearly similar.

The results from neither of the two resting stages studied are in agreement with those of Sax and Swanson (6) where the sensitivity was shown to be nearly the same on a per-cell basis, and much lower for the tetraploid on a per-chromosome basis. Their study was made at a stage 76 hr. previous to the prophase of the pollen grain division—a stage where the sensitivity was possibly influenced (as was found in the second part of the present study) by the difference in the speed of development in the haploid and diploid cells.

The most reasonable conclusion from the present research would seem to be that the true sensitivity is best indicated by the results of the analysis of the pollen grain division. At the resting stage when these cells were X-rayed there can be no doubt of the uniformity of stage, both within the anthers of a single bud and between the diploid and tetraploid material. Under these conditions the diploid and tetraploid chromosomes were shown to be of equal sensitivity to X rays, and any differences in the susceptibility of the cells or the plant as a whole must be interpreted as due to a change in the number of chromosomes, not to any basic change in the nature of the cell itself.

The results of other research based on the survival of diploid and tetraploid seedlings are not contradictory to this hypothesis. For the X-ray effect to be lethal to a seedling there must be a deletion of the same genes from homologous chromosomes. Since there are four homologues in a tetraploid compared with only two in a diploid species, there is less chance that a series of X-ray breaks will be lethal to the tetraploid, and the seedlings are thus able to survive higher dosages.

In a similar way it is logical to expect that intermixed diploid and polyploid cancer cells can be differentially killed by treatment with X radiation. By the elimination of the more sensitive diploid cells an abnormal growth may become mainly composed of the more resistant polyploid cells. This may account, at least in part, for the familiar development of radioresistance typical of many types of tumors following irradiation. Indeed, under certain conditions polyploid cells may even be induced by irradiation (Sparrow (9)).

If, as is indicated by the results of this research, diploid and tetraploid chromosomes are of equal sensitivity, then any difference in the reaction of the

cells must be due to the difference in the number of chromosomes they contain. Considering this cellular reaction mathematically, it means that for man the chances of a lethal effect being produced in a diploid body cell are $24x^2$, while for a tetraploid cell they are $24x^4$, where x is the percentage frequency of breaks which could cause a lethal effect. For death to occur in all cells of a diploid tissue the chromosome breakage frequency necessary may be calculated to be 20.4%, while in tetraploid tissue 45.2% is required to produce a similar effect.

Superimposed upon this difference is the variation in sensitivity during the cell division cycle and the variation in the rate of the mitotic cycle in diploid and polyploid tissue. However, the fact of equal sensitivity in the chromosomes is fundamental to a study of these other variables.

Acknowledgments

I wish to acknowledge the valuable technical assistance of Miss Ann Woodworth in the work involving the pollen grain mitosis, and of Miss Joan McGowan in the work on the pollen tube division. I wish also to thank Prof. Karl Sax and Dr. A. H. Sparrow for helpful suggestions in the preparation of the manuscript.

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THE EFFECT OF SALTS ON THE GROWTH OF *MICROCOCCUS HALODENITRIFICANS* N. SP.[1]

By J. Robinson[2] and N. E. Gibbons

Abstract

A halophilic coccus, *Micrococcus halodenitrificans* n. sp., isolated from meat curing brines is described. This organism grows optimally in media containing between 4.4 and 8.8% sodium chloride as determined by viable counts and manometric methods. The viable count decreases in media containing 2.2% or less sodium chloride. As salt concentrations increase above 8.8%, the length of the lag phase increases and the rate of growth decreases. The organism exhibits a specific sodium chloride requirement for growth. However, it continues to respire in the presence of sodium bromide.

Introduction

Although the effects of salts on the growth of halophilic bacteria have usually been studied qualitatively (4, 9) some quantitative evaluations, such as the rate of deamination (1), the rate of oxidation of sodium thiosulphate (8), and the time to bring about the decomposition of cellulose (7), have been made.

In this report the effect of a number of salts on the growth of a micrococcus from meat-curing brines has been determined qualitatively by visual evaluation of broth culture density. The effect of sodium chloride concentration has been measured directly by changes in the viable cell content of liquid cultures, and indirectly by the increase in oxygen consumption in a Warburg respirometer during successive observation periods.

Methods

The micrococcus was carried routinely on a basal medium containing 0.5% proteose peptone (Difco) and 0.5% tryptone (Difco) to which 3.5% sodium chloride had been added. This medium is referred to as 3.5% salt agar or broth. Cultures were incubated at 25°C. unless otherwise stated.

Viable populations were determined by the "drop plate" procedure (2) on agar containing 3.5% salt.

Manometric studies were made at 27°C. using a slight modification of the method of Greig and Hoogerheide (5). Inoculum and 0.1 *M* phosphate buffer containing appropriate concentrations of salt were added aseptically to Warburg vessels. Inocula were prepared by suspending the organisms from a 24 hr., 3.5% salt agar culture in 0.1 *M* phosphate – 3.5% sodium chloride

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Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Part of a thesis submitted by the senior author to the Faculty of Graduate Studies and Research, McGill University, in partial fulfillment of the requirements for the degree of Ph.D. Issued as N.R.C. No. 2661.

[2] *Now with the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa.*

buffer. The cell suspensions were passed through a wad of sterile cotton to remove debris, washed twice in salt buffer solution, and finally adjusted aseptically to a fixed nephelometer value. Preliminary studies indicated that the optimum pH was 7.6 and the optimum concentration of substrate 3.5% each of proteose peptone and of tryptone. The substrate was tipped from the sidearm into the reaction flask (at the rate of 0.1 ml. of substrate per 3.0 ml. of total solution) 10 min. after initiation of the test, as it had been shown that the time of mixing of substrate and inoculum had little effect on the oxygen consumption of the growing cells.

Test solutions were added in a random manner to the reaction flasks and these in turn were randomized in the water bath. The results are the average of duplicate readings replicated three times on different days.

Results

Effect of Various Salts on Growth

Basal broth containing three concentrations of a wide variety of salts was loop-inoculated from a 24 hr. culture in 3.5% salt broth and examined for growth after 15 days at 25°C. From the results (Table I) it is evident that the micrococcus grew only in the presence of sodium chloride. The micrococcus survived in solutions of several of these salts for some time as indicated by growth when transferred to broth containing 4.4% sodium chloride. The organism was still viable after 20 days in 0.8 and 1.6 *M* sodium chloride. In 0.4 *M* sodium chloride however survival depended on the size of the original inoculum; a few organisms survived when heavy inocula were used. In 1.6 *M* lithium chloride, the organisms were still viable at eight days but not at 14 days. The coccus also survived fairly well in the higher concentrations of sodium bromide and sodium nitrate.

The Effect of Sodium Chloride Concentration on Growth

Twenty-four-hour broth cultures were diluted 10,000-fold in 3.5% salt broth and inoculated at the rate of 0.1 ml. of inoculum per 100 ml. of culture medium into the basal broth containing 0, 0.55, 1.1, 2.2, 4.4, 8.8, 11.7, 17.6, and 23.4% sodium chloride (0, 0.09, 0.187, 0.375, 0.75, 1.5, 2, 3, and 4 *M*). Under these conditions the organism did not survive in media containing 2.2% salt and less (Fig. 1). In the growth and survival studies reported in the preceding section slight growth was obtained in 0.4 *M* (2.3%) sodium chloride when an inoculum of 0.1 ml. of a 48 hr. culture was used as the inoculum. No growth or survival was obtained with a loop-inoculum of the same culture. Even in 0.5 *M* sodium chloride this effect of size of inoculum was still noticeable; growth was detected in 48 hr. with the heavier inoculum but not until 72 hr. with the lighter inoculum. A concentration of about 2.2% sodium chloride therefore seems to be the limiting value, slight growth taking place if enough cells are present but a decrease without recovery taking place if only a few cells are transferred.

TABLE I

THE EFFECT OF A NUMBER OF SALTS AT 0.4, 0.8, AND 1.6*M* CONCENTRATION ON THE GROWTH AND SURVIVAL OF *M. halodenitrificans*

Salt	pH range	Growth after 15 days at 25°C.			Survival after:											
					24 hr.			48 hr.			120 hr.			144 hr.		
		0.4	0.8	1.6	0.4	0.8	1.6	0.4	0.8	1.6	0.4	0.8	1.6	0.4	0.8	1.6
Sodium chloride	6.5—6.6	±	+	+	±	+	+	±	+	+	±	+	+	±	+	+
Lithium chloride	6.3—6.5	−	−	−	+	+	+	−	+	+	−	+	+	−	+	+
Sodium bromide	6.5—6.6	−	−	−	+	+	+	+	+	+	−	+	+	−	+	+
Sodium nitrate	6.5—6.6	−	−	−	+	+	+	+	+	+	−	+	+	−	−	+
Magnesium chloride	6.0—6.4	−	−	−	+	+	+	−	+	+	−	−	−	−	−	−
Ammonium chloride	5.8—6.1	−	−	−	+	+	+	−	+	+	−	−	−	−	−	−
Potassium chloride	6.7	−	−	−	+	+	+	−	−	+	−	−	−	−	−	−
Potassium bromide	6.7	−	−	−	+	+	+	−	−	+	−	−	−	−	−	−
Sodium sulphate	7.2	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Sodium citrate	7.2	−			−			−			−			−		
Potassium sulphate	6.8	−	−		+	−		−	−		−	−		−	−	
Sodium iodide	6.6	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Potassium iodide	6.8—7.0	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
Sodium ammonium phosphate	7.5	−	−	−												
Ammonium sulphate	6.4—6.8	−	−	−												

Since this organism requires a minimal concentration of 2.2% sodium chloride and will not grow in the presence of other salts it may be classed as a true halophile.

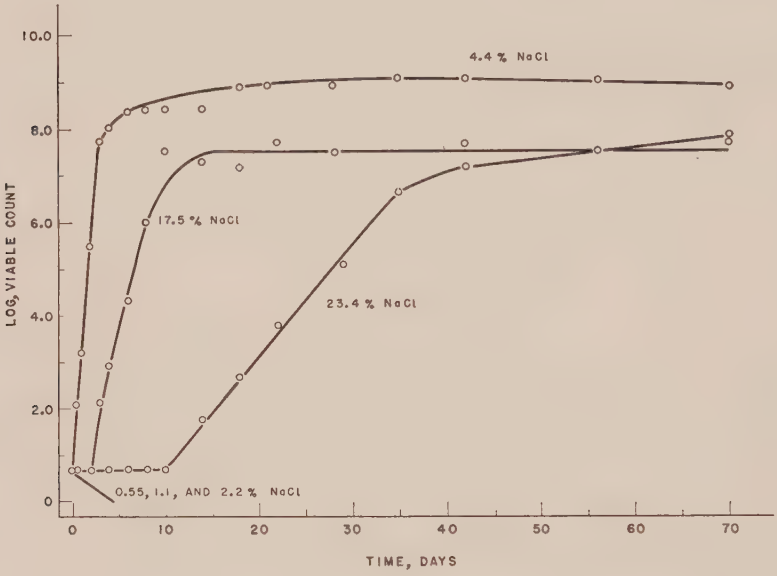

FIG. 1. Effect of salt on growth of *M. halodenitrificans*.

Maximum rate of growth and cell yield was obtained at a concentration of 4.4%. At higher concentrations the rate of growth decreased and the length of the lag phase increased. As the salt concentration was increased, the length of the lag phase increased from four to six hours in 4.4% salt to almost 14 days in 23.4% salt (Fig. 2).

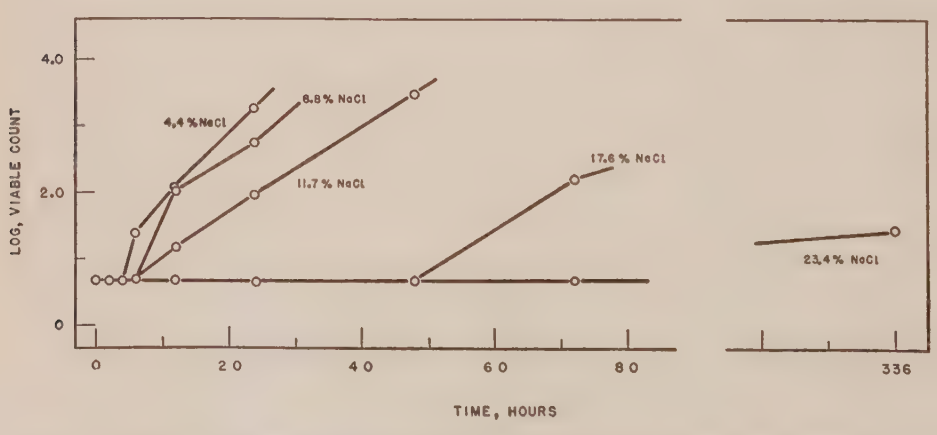

FIG. 2. Effect of increasing salt concentration on the lag phase of *M. halodenitrificans*.

In the Warburg respirometer the micrococcus consumed very little oxygen when suspended in the basal medium containing 0.55% salt (Fig. 3). However, in both 4.4 and 8.8% salt the amount of oxygen consumed increased

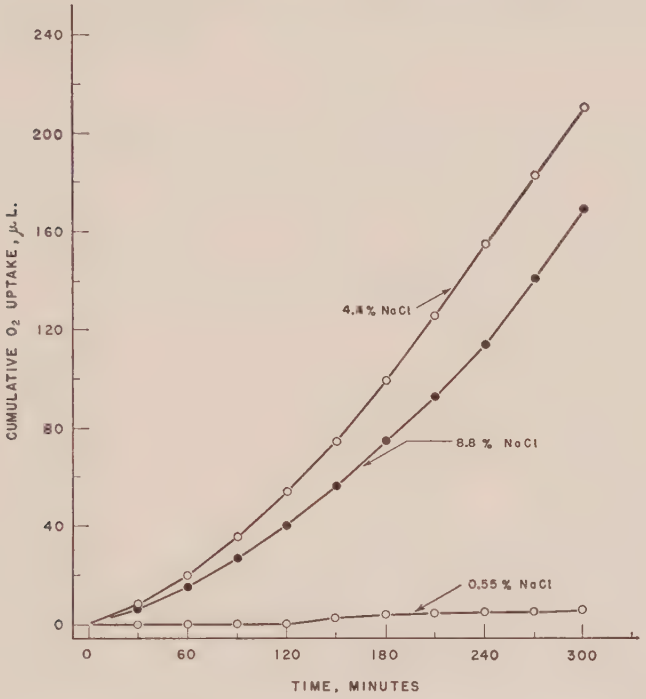

FIG. 3. Effect of salt on cumulative oxygen uptake of cells of *M. halodenitrificans*.

during successive 30 min. observation periods as is characteristic of growing bacteria. Some proliferation occurred at both of these concentrations (Table II) although the initial number of cells was very high.

TABLE II

THE VIABLE CELL COUNT OF *M. halodenitrificans* AND *P. denitrificans* AFTER A 300-MIN. INCUBATION PERIOD IN THE WARBURG RESPIROMETER

Per cent NaCl in vessels	Log number of organisms per ml.*	
	M. halodenitrificans	*P. denitrificans*
0	—	8.4
0.55	4.9	7.9
1.1	7.1	6.6
2.2	7.9	6.7
4.4	8.4	5.0
8.8	8.4	<3.0
17.6	8.0	—

**Initial count log 7.9 and 8.0 per ml. respectively.*

Maximum oxygen consumption in the five hour observation period was evident in 4.4% salt (Fig. 4). Although more oxygen was consumed in 2.2% salt than in 17.6% proliferation did not take place at either concentration

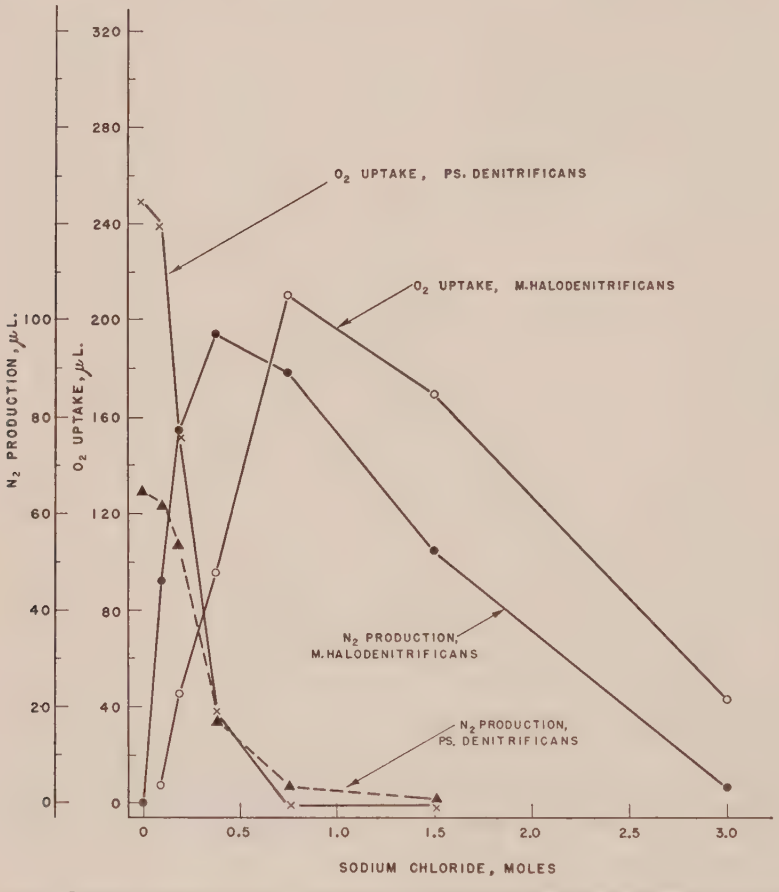

FIG. 4. Oxygen uptake and N_2 production of cells of *M. halodenitrificans* and of *P. denitrificans* in salt media during a five hour observation period.

during the period of observation. Moreover, cells exposed to 17.6% salt remain viable and will eventually multiply, whereas cells exposed to 2.2% salt for any length of time will usually not divide (Fig. 1).

In contrast, with the nonhalophilic *Pseudomonas denitrificans*,* oxygen consumption and nitrate reduction decreased with increasing salt concentration (Fig. 4). The viable cell count also decreased with increasing salt concentration (Table II).

With the halophilic coccus, nitrogen production from nitrite was optimum at 2.2% salt, slightly below the optimum salt concentration for respiration and for growth (Fig. 4). This point will be considered in more detail in a later paper (6).

*Culture kindly supplied by Dr. Sacks, Division of Plant Nutrition, University of California.

Effect of Sodium Bromide on Oxygen Consumption

Cells of the micrococcus were inoculated into media containing 0.55 and 4.4% sodium chloride and 7.7% sodium bromide (approximately equimolar with 4.4% sodium chloride) and the oxygen consumption measured over a five hour period (Table III). As noted before (Fig. 3), in 0.55% salt a small

TABLE III

THE OXYGEN UPTAKE IN μLITERS OF *M. halodenitrificans* IN 0.55 AND 4.4% SODIUM CHLORIDE AND IN 7.7% SODIUM BROMIDE

Oxygen uptake during:	NaCl		NaBr
	0.55% 0.09*M*	4.4% 0.75*M*	7.7% 0.75*M*
First hour	1.7	8.0	4.2
Second hour	2.3	7.0	6.8
Third hour	3.5	9.6	8.4
Fourth hour	0.9	15.0	7.8
Fifth hour	1.7	23.0	9.0
Total	10.1	62.6	36.2

and fairly constant amount of oxygen was consumed during each hour indicating a dying culture. In 4.4% salt the oxygen consumption increased with each successive period, the characteristic of a growing culture (3). In the presence of sodium bromide an appreciable and slightly increasing amount of oxygen was consumed each time indicating a "resting" culture. This suggests that sodium bromide cannot satisfy the growth requirements of the organism but at least maintains it in a viable state for some time.

Description of the Organism

The organism was isolated on 10% salt agar from spent Wiltshire bacon curing brine. It is a Gram-negative coccus, 0.5 μ in diameter, occurring singly or in pairs. Sodium chloride is necessary for growth and the organism dies in salt concentrations of 2.2% and less. The coccus grows optimally in media containing between 4.4 and 8.8% sodium chloride; above 8.8% salt the length of the lag phase increases and the rate of growth decreases. The organism exhibits a specific sodium chloride requirement and will not grow in the presence of a number of other sodium and potassium salts. Salt concentration has little effect on the morphology of the coccus.

The following cultural characteristics were observed in media containing 3.5% sodium chloride:

Colonies on agar are cream-colored, glistening, opaque, convex, circular, entire, and butyrous. Gelatin is liquified but milk is not visibly altered. Indol and hydrogen sulphide are not produced; catalase activity is present but urease activity is absent. Acid is not produced from glucose. Nitrate and

nitrite are reduced to molecular nitrogen. The pH increases during denitrification, presumably because of release of sodium ions, since ammonia is not produced (6).

The organism is aerobic or facultatively anaerobic. It grows rapidly in peptone medium but no entirely synthetic medium that will support growth has been found.

No description of a denitrifying halophilic micrococcus has been found in the literature. This organism has therefore been designated *Micrococcus halodenitrificans* n. sp.

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THE EFFECTS OF SALTS ON THE NITRITASE AND LACTIC ACID DEHYDROGENASE ACTIVITY OF *MICROCOCCUS HALODENITRIFICANS*[1]

By J. Robinson[2]

Abstract

With *Micrococcus halodenitrificans*, maximum production of nitritase occurs just after nitrite has been reduced completely to nitrogen. Maximum denitrifying activity takes place at pH 7.6 in the presence of 0.38 to 0.75 molar sodium chloride or sodium bromide. The activity is slightly less in the presence of lithium chloride but reaches a peak at 0.19 to 0.38 molar concentration. The reduction of nitrate to nitrite is inhibited by azide whereas reduction of nitrite to nitrogen is not, suggesting the possibility that at least two separate enzymes may be involved in these reactions. The lactic acid dehydrogenase of this organism shows maximum activity in the presence of 0.75 molar sodium chloride.

Introduction

The denitrification of nitrate or nitrite to nitrogen proceeds anaerobically in the presence of nitrate or nitrite reductase, a suitable hydrogen donor, and the appropriate dehydrogenase (1, 2, 3, 4, 6, 9, 11). At least two reductases are involved in the conversion of nitrate to ammonia. Lascelles and Still (3) have shown that *M*/1000 hydrogen cyanide and *M*/500 sodium azide inhibit the reduction of nitrate to nitrite but have no effect on the nitrite–hydroxylamine conversion. Woods (11) has shown that maximum reduction of nitrate, nitrite, and hydroxylamine takes place at pH 7.0 to 7.8, pH 6.4 to 6.8, and above pH 8.0 respectively. Sacks and Barker (8) indicate that oxygen suppresses the production of nitrite reducing enzymes more than those reducing nitrate to nitrite.

Micrococcus halodenitrificans (7) is capable of reducing nitrate completely to nitrogen in two, four to six, and seven to 10 days in broth containing 3.5, 11.7, and 17.5% sodium chloride respectively. In this paper the effects of sodium chloride and other salts on the nitrite–nitrogen reductase system are presented, together with some studies on the lactic acid dehydrogenase of this organism.

Method

Enzyme activity in resting cell suspensions was determined manometrically at 27°C. using the technique and randomization methods described previously (7).

For evaluation of nitritase and nitratase activity the main chamber of the Warburg flasks contained 1.0 ml. of 0.1 *M* buffer–salt solution, 1.0 ml. of a

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Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, and the Department of Bacteriology, Macdonald College, Macdonald College P.O., Que. Part of a thesis submitted to the Faculty of Graduate Studies and Research, McGill University, in partial fulfillment of the requirements for the degree of Ph.D. Issued as N.R.C. No. 2666.

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0.1 *M* solution of a suitable hydrogen donor in appropriate concentrations of salt, 0.4 ml. of distilled water, and, as a source of enzyme, 0.5 ml. of a washed cell suspension of *M. halodenitrificans* in 3.28% sodium chloride solution. The sidearm contained 0.1 ml. of 0.1 *M* sodium nitrite (or 0.1 ml. of 0.1 *M* sodium nitrate for nitratase determination) and the center well 0.2 ml. of 10% potassium hydroxide. The flasks were flushed with nitrogen and the rate of nitrogen production followed.

For lactic acid dehydrogenase activity a second milliliter of buffer–salt mixture replaced the hydrogen donor in the main chamber of the flask and 0.1 ml. of 0.1 *M* sodium lactate was used in the sidearm. After agitating for 10 min. to bring the flasks to equilibrium, measurements of oxygen uptake were made at 10-min. intervals.

Unless otherwise stated, all manometric results are given as averages of readings duplicated on one day, and represent the gas exchange effected by 2.0 mgm. of cells during a 30 min. observation period.

Nitrate and nitrite were determined chemically by the methods of MacDougall and Roadhouse (5) and of White (10) respectively.

Results

Nitritase Production During Growth

To determine at what stage of culture development maximum enzyme production occurred, 300-ml. portions of proteose peptone – tryptone broth containing 11.7% sodium chloride and 0.085% sodium nitrate were inoculated with 0.5 ml. of a broth suspension of the micrococcus. Flasks were removed at random after 30, 60, 72, and 90 hr. incubation at 70°F., the cells harvested by centrifugation, and nitrate and nitrite determined on the supernatant fluid. The cells were washed three times in a 3.28% sodium chloride solution and finally suspended in a salt solution of the same concentration. The nitritase activity of these cells was determined in the presence of 2.2% sodium chloride within two hours of harvesting.

Nitritase activity of 2.0 mgm. of cells obtained from the 30-hr. broth culture was negligible during a 30 min. observation period (Fig. 1). Enzyme activity then increased very rapidly in cultures incubated 60 and 72 hr. during which time the nitrite content of the broth was reduced to zero. Eighteen hours later the nitritase activity had already begun to show a sharp decline. Maximum enzyme production occurred just after the nitrite had been reduced completely to nitrogen.

Cells grown on the surface of agar had from a half to a third the nitritase activity of cells grown in broth. Whether this was because of the inhibitive action of oxygen (8) or because the peak of enzyme production had not been reached is not known.

Effect of Storage of Cells

To determine the effect of salt concentration and time of storage of cells on their enzyme activity, cells were grown in broth containing 3.5% salt and equal quantities of sedimented cells suspended in 1.0% and in 3.28% sodium

chloride solutions. The viable population and nitritase activity of these suspensions were determined initially and after four and six days' storage at 5°C. Enzyme activity was determined in solutions containing 0.55 and 2.2% sodium chloride.

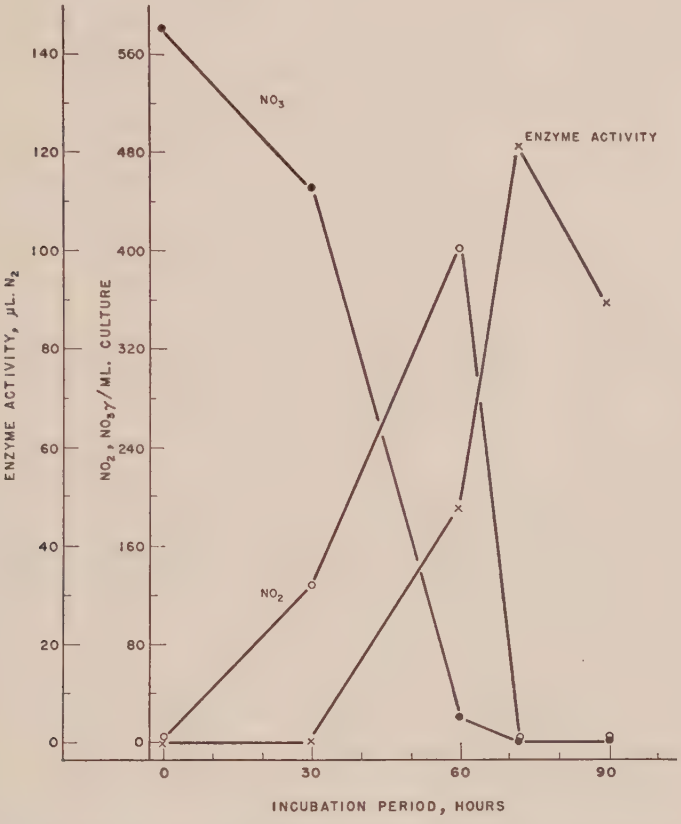

FIG. 1. Nitritase formation in relation to nitrate and nitrite reduction in a culture of *M. halodenitrificans*. Enzyme activity expressed as μliters of nitrogen produced in 30 min. by 2.0 mgm. cells.

The nitritase activity of cells suspended in 1.0% salt was always lower than that of cells suspended in the more concentrated solution (Table I). Both the activity and viability of the cells suspended in 1.0% salt decreased rapidly. The viability of the cells suspended in 3.28% sodium chloride remained fairly constant although their activity diminished somewhat during the six day storage period.

Effect of Buffer and pH

Nitritase activity was assessed in phosphate and in borate buffers adjusted to pH 6.0, 7.0, and 8.0 and to pH 8.0, 9.0, and 10.0 respectively. All buffers contained 2.2% sodium chloride. For the respective pH values, nitritase

TABLE I

THE EFFECT OF SALT CONCENTRATION AND STORAGE TIME ON THE NITRITASE ACTIVITY AND VIABILITY OF CELLS OF *M. halodenitrificans*

(Nitritase activity — μliters N_2 per 30 min. per 2.0 mgm. cells)

Suspending medium	Storage time at 5°C., days	Nitritase activity		Viable cell count (log/ml.)
		0.55*	2.2*	
1.0% NaCl solution	Initial	44.1	66.8	9.8
	2	6.8	10.0	8.9
	6	8.1	4.8	7.9
3.28% NaCl solution	Initial	47.1	75.2	9.8
	2	39.6	53.6	9.7
	6	36.8	42.4	9.8

% NaCl in reaction vessels.

activity (μliters N_2 per 30 min. per 2.0 mgm. cells) in phosphate buffer was 12.8, 39.7, and 39.7, and in borate buffer 39.1, 30.1, and 11.0. Maximum activity took place in phosphate buffers at pH 7.0 and 8.0.

Effect of Hydrogen Donor

Molecular hydrogen or an organic compound, such as lactate, may operate as a hydrogen donor in conjunction with the nitrite–nitrogen reducing system. To determine which of several organic compounds could be associated with the reduction of nitrite by the halophilic micrococcus, nitritase activity was assessed in a sodium chloride concentration of 2.2% using 0.1 *M* solutions of several organic acids as potential hydrogen donors. Their activity (μliters N_2 per 30 min. per 2.0 mgm. cells) was as follows: lactic—80.6, acetic—52.0, tartaric—51.4, succinic—49.8, maleic—43.7, water control—37.0.

To associate possible hydrogenase activity of the halophilic micrococcus with its nitritase activity, reaction vessels were prepared with or without lactate and each with or without nitrite as substrate, in solutions containing 2.2% sodium chloride, and under atmospheres of hydrogen or nitrogen.

If the organism exhibited any appreciable hydrogenase activity, a negative rather than a positive gas pressure would be expected in the system containing sodium nitrite and cells under an atmosphere of hydrogen. In the presence of any hydrogenase activity, a considerably lower positive gas pressure would also be expected in a system containing lactate, cells, and nitrite under an atmosphere of hydrogen than in a comparable system under an atmosphere of nitrogen. Since approximately similar volumes of gas were produced in comparable systems under nitrogen and hydrogen (Table II), it is probable that cells of the halophilic micrococcus do not possess a hydrogenase system and that the nitrite reducing system of this halophile operates effectively in conjunction with a lactic acid oxidizing enzyme.

As a result of the preceding studies, the following procedure was adopted for the remaining experiments. Washed cell cultures incubated in salt–nitrate–nutrient broth until the nitrate and nitrite were completely reduced.

TABLE II

NITRITASE ACTIVITY OF *M. halodenitrificans* UNDER ATMOSPHERES OF HYDROGEN AND NITROGEN

(Nitritase activity — μliters N_2 per 30 min. per 2.0 mgm. cells)

Flask contents	Substrate	Nitritase activity under:	
		Hydrogen	Nitrogen
Buffer–lactate	Water	0	−1.9
	Nitrite	67.0	62.6
Buffer–water	Water	——	1.3
	Nitrite	30.6	26.8

The nitritase activity of these suspensions, after refrigeration for 18 hr. in 3.28% sodium chloride solution, was determined in phosphate buffer adjusted to pH 7.6, using sodium lactate as the hydrogen donor.

Nitritase as a Separate Enzyme

To determine whether nitrate and nitrite were reduced by the same or different enzymes, rate of nitrogen formation from these substrates was determined in the presence of 2.2% sodium chloride and various concentrations of sodium azide. Azide reduced nitratase activity but had little effect on nitritase activity (Table III). These results bear out the earlier observations of

TABLE III

THE EFFECT OF AZIDE AND IODOACETATE ON NITRITASE AND NITRATASE ACTIVITY

(μliters N_2 per 30 min. per 2.0 mgm. cells)

Inhibitor	Inhibitor concentration (moles)	Nitratase activity	Nitritase activity
Control	—	52.1	73.2
Sodium azide	10^{-2}	0.8	57.3
	10^{-3}	—	—
	10^{-4}	4.9	79.0
	10^{-5}	18.5	65.4

Lascelles and Still (3) that separate enzymes were responsible for the reduction of nitrate to nitrite and of nitrite to hydroxylamine. However, since *M. halodenitrificans* was unable to reduce hydroxylamine (at pH 7.6) it is assumed that the organism did not reduce nitrite by way of this compound. It is possible that hyponitrite, as suggested by Lloyd (4), is the more likely intermediate.

Effect of Sodium Chloride, Sodium Bromide, and Lithium Chloride on Nitritase Activity

M. halodenitrificans is unable to grow in media in which other salts have been substituted for sodium chloride although it survives in the presence of several salts for a number of hours (7). To determine the effect of these salts on nitritase activity, reaction vessels were prepared containing buffer–lactate solution to which equimolar concentrations of sodium chloride, sodium bromide, or lithium chloride had been added. The three salts were used in final molar concentrations of 0.09, 0.19, 0.38, 0.75, 1.5, and 3.0, corresponding to 0.55, 1.1, 2.2, 4.4, 8.8, and 17.6% sodium chloride. However, since the washed cell suspension of the test organism was made up in 3.28% sodium chloride, 0.09 molar sodium chloride was present in all flasks of sodium bromide and lithium chloride and their actual concentration was the difference between this amount and the values given above.

The nitritase activity of cells suspended in the various concentrations of sodium chloride and sodium bromide was practically identical and has not been differentiated (Fig. 2). Maximum activity was evident in concentrations between 0.38 and 0.75 *M*. With lithium chloride the maximum activity was between 0.19 and 0.38 *M* and at the higher concentrations nitritase activity was considerably less than with sodium chloride or bromide. This decreased activity was apparently not related to the higher osmotic pressure of the lithium chloride solution since a plot of nitritase activity against osmotic pressure gave curves practically identical in shape with those shown in Fig. 2.

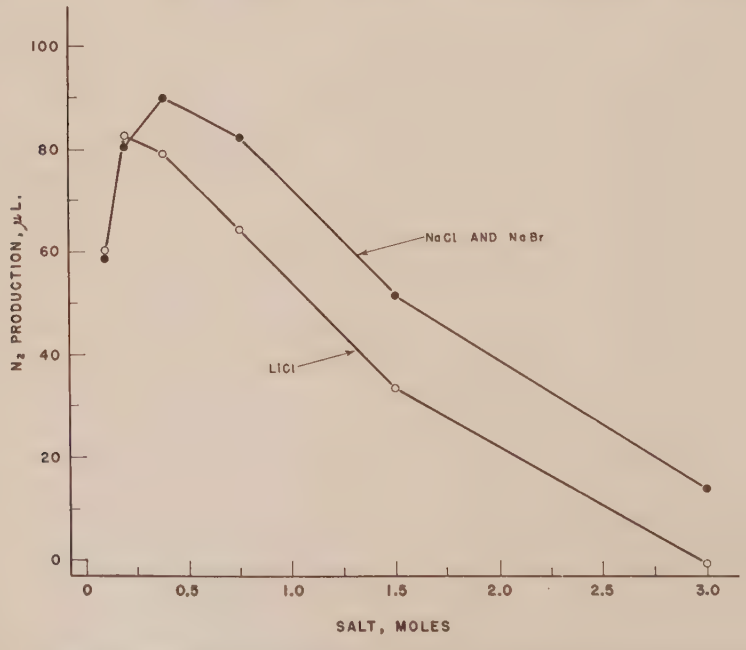

FIG. 2. Effect of equimolar concentrations of sodium chloride, sodium bromide, and lithium chloride on nitritase activity of cells of *M. halodenitrificans*.

The effect of concentration of sodium chloride on nitrite reduction is further shown in Fig. 3. Nitrogen was produced at a slightly diminishing rate at a salt concentration of 2.2% over practically the whole observation period,

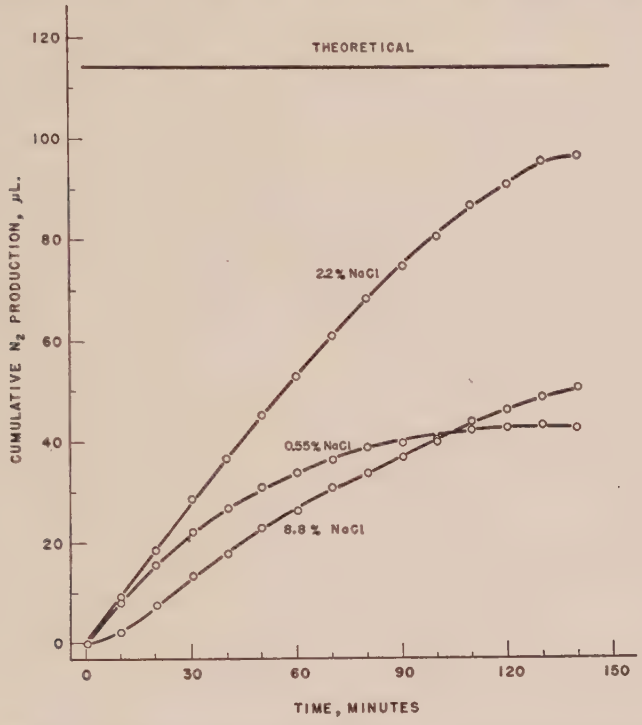

FIG. 3. Effect of sodium chloride on cumulative nitrogen production by *M. halodenitrificans.*

possibly because of an increase in pH of the medium. Under similar conditions the pH taken at 20-min. intervals from 0 to 120 min. increased as follows: 7.58, 7.63, 7.78, 7.90, 8.10, 8.70, 9.95. Ammonia is not produced under these conditions and the increase in pH is presumably due to release of sodium ion. As shown earlier, nitritase activity began to fall off at pH 9.0 and was sharply reduced at pH 10.0.

The marked reduction of activity after a short exposure to 0.55% salt was associated with plasmoptysis of the cells and was possibly the result of liberation of the enzyme (or perhaps a coenzyme) into the medium at suboptimal concentrations.

Effect of Sodium Chloride on Lactic Acid Dehydrogenase Activity

Preliminary experiments indicated that lactate was readily oxidized by the micrococcus, but glutamate, hypoxanthine, succinate, and malate were not. The effect of salt concentration on the lactic acid dehydrogenase system was studied manometrically using a washed cell suspension of *M. halodenitrificans* and the same salt–buffer solutions which had been used earlier for nitritase determinations.

The average results of duplicate runs replicated on different days are presented in Fig. 4. Lactic acid dehydrogenase and endogenous respiratory activity proceeded at a maximum rate in media containing 4.4% sodium chloride and decreased markedly in media containing higher or lower concentrations of salt.

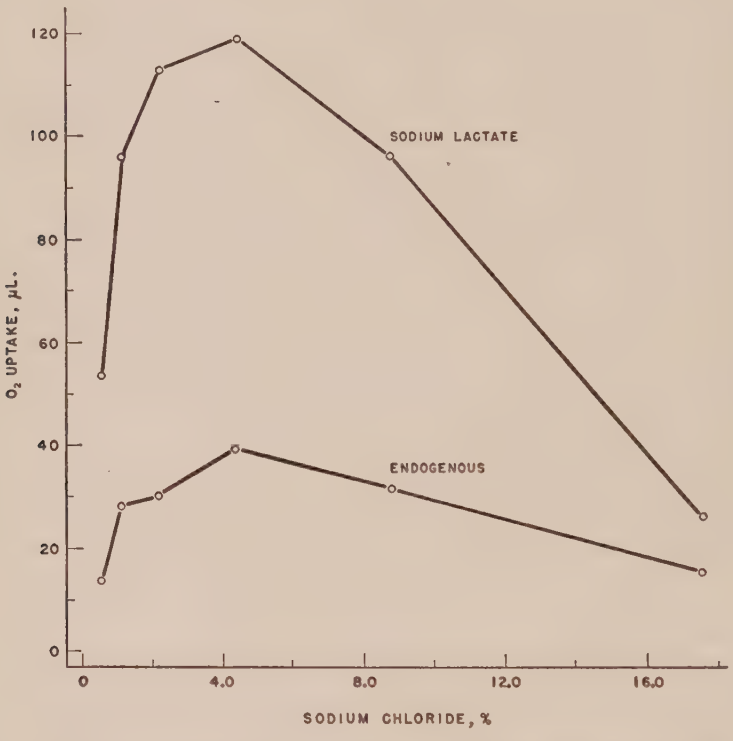

FIG. 4. Effect of sodium chloride on lactic acid dehydrogenase activity and endogenous respiration of cells of *M. halodenitrificans*. Activity expressed as µliters of oxygen consumed in 30 min. by 2.0 mgm. cells.

Discussion

Sodium chloride is required by *Micrococcus halodenitrificans* for growth, and at least two of the enzyme systems in resting cells showed maximum activity in the presence of this salt. The maximum activity of lactic acid dehydrogenase was in a medium containing about 4.4% salt, and of nitritase in media containing between 2.2% and 4.4% salt. Nitritase activity proceeded equally well in equimolar concentrations of sodium chloride and sodium bromide.

The nitrite–nitrogen nitritase system (as distinct from nitrite – hydroxylamine nitritase) is different from the nitrate–nitrite–nitrogen system since the latter was affected to a much greater degree by azide. The pathway of nitrite reduction is not known but it apparently does not proceed by way of hydroxylamine.

Micrococcus halodenitrificans possesses a mechanism capable of oxidizing lactic acid in conjunction with the reduction of nitrite. Since the organism was unable to oxidize hydrogen, or the sodium salts of maleic and succinic acids, these substances could not act as hydrogen donors during the course of nitritase activity.

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LONGEVITY OF SOME COMMON FUNGI IN CEREAL SEED[1]

By J. E. Machacek and H. A. H. Wallace[2]

Abstract

Tests made at regular intervals over a period of 10 years on naturally infested seed of wheat, oats, and barley that had been held in storage showed that most of the seed, while retaining its germinability, became free from viable fungi before the end of the storage period. *Alternaria tenuis sensu* Wiltshire in all three crops, *Helminthosporium sativum* P.K. & B. in wheat and barley, and *Septoria nodorum* Berk. in wheat died out comparatively rapidly. *H. avenae* Eidam in oats and *H. teres* Sacc. in barley lost their viability slowly and since, by the end of the storage period, most of the microorganisms associated with them in infested kernels were dead, these two fungi could generally be recovered in pure culture.

Introduction

Seed of cereal crops produced in Canada has been found to carry internally a wide variety of fungi (4, 5, 7). Most of these fungi seem to be nonpathogenic, but some of them are known to cause plant diseases of considerable importance.

Other workers have found that some species of fungi may live for a number of years in cereal seed. For instance, Christensen (1) found *Helminthosporium sativum* P.K. & B. in barley seed to be alive after seven years. *H. gramineum* Rabenhorst was found by Leukel *et al.* (6) to live in barley seed for five years and by Shands (9) for 10 years. According to Paxton (8), the latter fungus lived in a herbarium specimen for as long as 16 years. Other data from Shands (9) show that *Gibberella saubinetii* (Mont.) Sacc. lived for only 27 months and *Fusarium culmorum* (W. G. Sm.) Sacc. and *F. avenaceum* (Fr.) Sacc. for an even shorter period. However, he recovered a species of *Alternaria* from barley seed even after a storage period of 75 months.

In the present study, an attempt was made to determine the longevity in cereal seed of a very common and seemingly innocuous species, *Alternaria tenuis sensu* Wiltshire, and of four common and important pathogens, *Helminthosporium sativum*, *H. teres* Sacc., *H. avenae* Eidam, and *Septoria nodorum* Berk. The project was initiated in 1940 by Dr. F. J. Greaney of this laboratory. After his resignation in 1946, the present writers continued work on the project until its completion in 1950.

Materials and Methods

Twenty-two lots of seed, including 11 lots of wheat, four lots of oats, and seven lots of barley, were selected for the investigation. Of the 11 lots of wheat seed, two of the variety Pentad were heavily infested with *A. tenuis;*

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Contribution No. 1134 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Canada.

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one each of the varieties Pentad and Regent was heavily infested with *H. sativum;* three of Pentad and one each of Renown and Mindum were moderately infested with mixed *A. tenuis* and *H. sativum;* and one each of Thatcher and Red Fife was moderately infested with *S. nodorum.* Of the four lots of oats, one of the variety Vanguard was heavily infested with *A. tenuis;* and two of the variety Erban and one of the variety Banner were heavily infested with *H. avenae.* Of the seven lots of barley, three of the variety Charlottetown 80 were heavily infested with *H. sativum*; two of the variety Hannchen were moderately infested with mixed *A. tenuis* and *H. sativum*; one of variety O.A.C. 21 was moderately infested with *H. teres*, and one was moderately infested with mixed *H. teres* and *H. sativum.* Other fungi occurred in these lots of seed, but in negligible amounts.

The seed was stored in heavy Manila-paper envelopes in a galvanized metal box provided with a loosely fitting lid to permit aeration and afford protection from rodents. The box of seed was kept the year round in an unheated dry shed, except for short periods at three-month intervals when it was removed to the laboratory to obtain samples for testing.

For purposes of this test, 100 seeds from each lot were first surface-sterilized by immersion in a 1:3 mixture of 95% ethyl alcohol and 0·1% solution of mercuric chloride in water, the duration of immersion being four minutes for wheat, two minutes for oats, and five minutes for barley. After surface-sterilization the seed was washed in three changes of sterile water and transferred to Petri dishes, each one containing 15 cc. of potato–sucrose agar acidified with lactic acid (0.5 cc. of 10% lactic acid to each 150 cc. of agar). The dishes were incubated at a temperature varying from 65° to 75° F. for a week, after which the fungus colonies emerging from the seeds were examined and counted.

A count was made also of germinating seeds. During the first part of the investigation, the percentage germination was obtained from seed sown in soil in the greenhouse. Later, when the supply of seed became low, the percentage germination was obtained from the plate cultures just mentioned.

Experimental Results

Longevity of Fungi in Lightly Infested and in Heavily Infested Seed

As the lots of seed studied contained widely different percentages of infested kernels, an attempt was made to determine whether the decline in infestation occurred at the same rate in lightly infested and heavily infested lots of seed. Special lots of seed were chosen for this comparison. The initial infestation in the seed, expressed as the average infestation in three samples of each kind, was as follows: wheat (*A. tenuis*) 15.6% and 83.3%; wheat (*H. sativum*) 13.6% and 59.0%; barley (*A. tenuis*) 9.0% and 69.0%; barley (*H. sativum*) 13.6% and 93.3%.

To permit a direct comparison between the decline of infestation in lightly infested seed and that in heavily infested seed, the number of colonies of *A. tenuis* and *H. sativum* observed at each plating after the first was converted

into a percentage of the number found at the first plating. It was found that, in both wheat and barley, both fungi declined as rapidly in lightly infested seed as in heavily infested seed (Fig. 1).

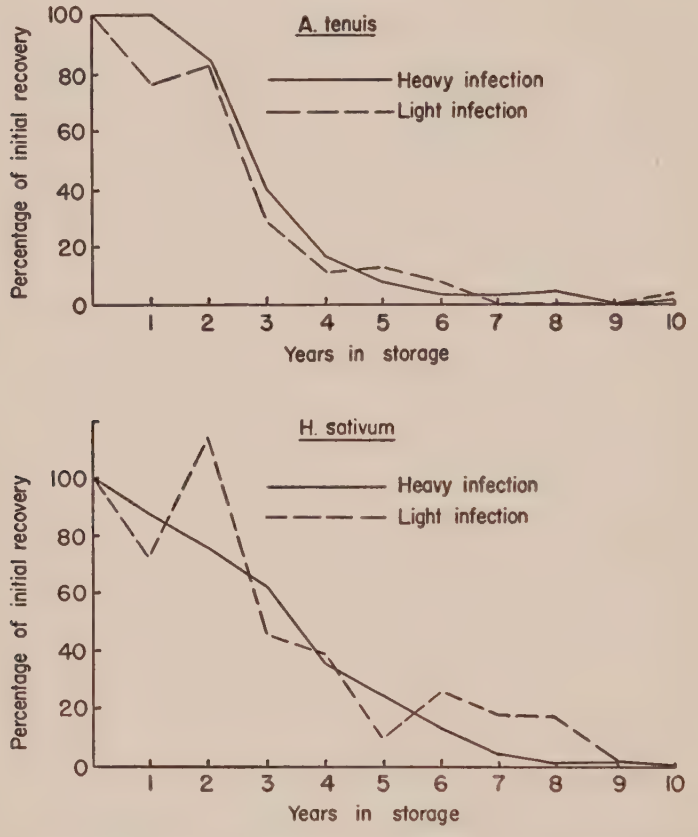

Fig. 1. Longevity of *Alternaria tenuis* and *Helminthosporium sativum* in lightly infested and in heavily infested wheat seed.

Longevity in Seed of Different Species of Fungi

As it appeared, from the somewhat limited evidence just presented, that the percentage infestation by *A. tenuis* and *H. sativum* was not related to the longevity of these fungi in seed, it seemed permissible to compare the rate of decline for other species of fungi infesting cereal seed without considering the percentage of infested kernels. On this basis, therefore, the following comparisons were made: between *A. tenuis*, *H. sativum*, and *S. nodorum* in wheat seed; between *A. tenuis* and *H. avenae* in oat seed; and between *A. tenuis*, *H. sativum*, and *H. teres* in barley seed.

In wheat, there was not a great difference between the three fungi in their rate of decline (Fig. 2), although for *S. nodorum* the rate was slightly more rapid than for the other two fungi during the first year of storage, and no

seeds yielded this fungus after the seventh year. Under the same conditions *H. sativum* lived for nine years and a small percentage (1.6%) of *A. tenuis* was found even after 10 years.

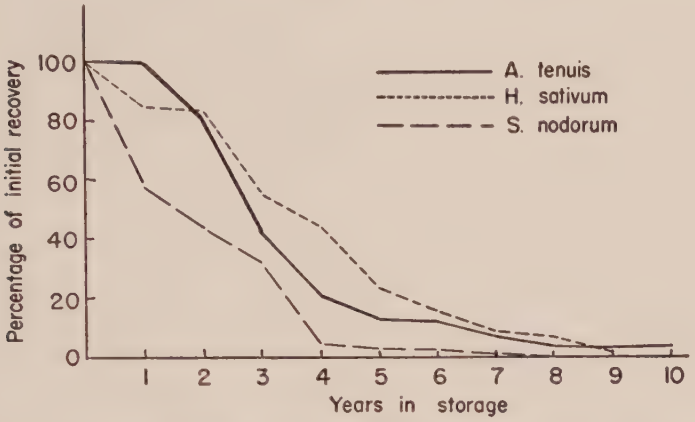

Fig. 2. Longevity of *Alternaria tenuis*, *Helminthosporium sativum*, and *Septoria nodorum* in naturally infested wheat seed.

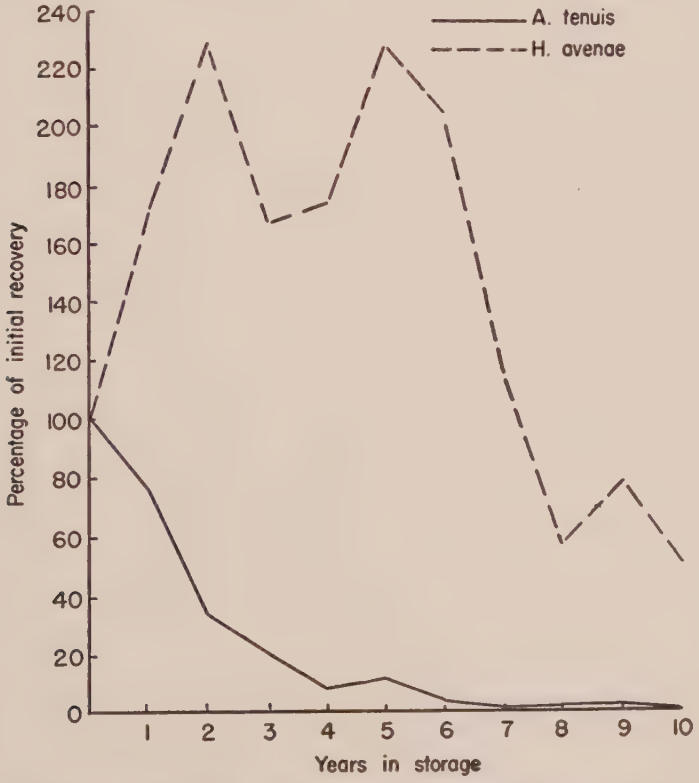

Fig. 3. Longevity of *Alternaria tenuis* and *Helminthosporium avenae* in naturally infested oat seed.

In oats, the rate of decline for *A. tenuis* was different from that for *H. avenae* (Fig. 3). The decline of *A. tenuis* was about the same as in wheat, except that in oats it was greater during the first year of storage. The progress of decline of *H. avenae*, however, was distinctly different from that of any of the other three fungi just mentioned. During the first five years of storage *H. avenae* was recovered from a higher percentage of seeds than at the commencement of the experiment, although the percentages in the third and fourth years were considerably lower than in the second and fifth years. After the fifth year there was a definite decline in *H. avenae* and, during this period many of the seeds were found to be infected solely with this fungus. At the end of 10 years of storage, none of the oat seeds yielded *A. tenuis* but 10% of them still yielded *H. avenae*.

The decline of *A. tenuis* and *H. sativum* in barley was similar to that found in wheat. *H. teres*, however, increased during the second and third years of storage, reaching a maximum in three years and thereafter declining (Fig. 4).

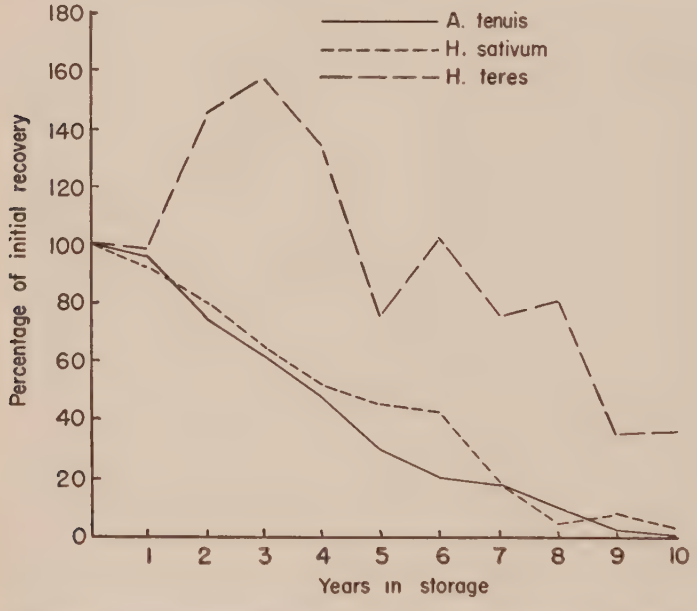

Fig. 4. Longevity of *Alternaria tenuis*, *Helminthosporium sativum*, and *Helminthosporium teres* in naturally infested barley seed.

In this respect its behavior resembled that of *H. avenae* in oats. At the end of 10 years of storage, 0.6% of the seeds yielded *A. tenuis*, 1.7% yielded *H. sativum*, and 3.1% yielded *H. teres*.

No reason can be given to account for the increase in the percentages of seeds yielding *H. avenae* and *H. teres* during approximately the first half of the 10 year storage period. It is suggested, however, that during the first few years of storage there may have been present on or in the seed saprophytic microorganisms that were antagonistic to *H. avenae* and *H. teres*. These

organisms would prevent or retard the growth of the two fungi and thus interfere with their detection. Later, when they had died out, *H. avenae* and *H. teres* would develop unimpeded.

Viability of Infested Seed After 10 Years of Storage

Much has been written concerning the viability of seed held in storage and most of the information has been summarized by Crocker (2). There seems to be, however, very little information in the literature concerning the longevity of cereal seed infested with fungi. Some information on this subject was provided by Dungan and Koehler (3) who investigated the decline in viability in five lots of maize seed, each lot infested with a different fungus. They found that, while all the seed became free of viable fungi by the end of nine years of storage in their laboratory, it germinated quite well even after 10 years.

In the present investigation, it was found that in 11 lots of wheat seed, after 10 years of storage, the germination ranged from 18% to 85% (av. 66%). In oats the range for four lots was from 88% to 96% (av. 92%), while for seven lots of barley it was 62% to 98% (av. 83%). When the lots of seed were grouped according to the kind of fungus that infested the seed, it was found that the mean germination of wheat seed infested principally with *A. tenuis* was 51%; of wheat seed infested with *H. sativum* 54%; and of wheat seed infested with *S. nodorum* 60%. The mean germination of oat seed infested with *A. tenuis* was 96%; and of oat seed infested with *H. avenae* 92%. For barley seed infested with *A. tenuis* the mean germination was 71%; for barley seed infested with *H. sativum* 86%; and for barley seed infested with *H. teres* 54%.

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THE MICROSCOPIC WOOD STRUCTURE OF NORTH AMERICAN SPECIES OF *CHAMAECYPARIS*[1]

By M. W. Bannan[2]

Abstract

Trends in variation in different parts of the tree with regard to such anatomical features as tracheid and ray cell dimensions, size and distribution of rays, size and arrangement of pits, and thickness of cell walls resemble those recorded for other Cupressaceae. Comparative data relating to homologous wood samples show slight differences in mean values between the three American species of *Chamaecyparis*, but the intraspecific variability is usually so extensive that the specific ranges overlap widely. No single microscopic character seems completely reliable for diagnostic purposes, but certain structural features are valuable when used together. These are the frequency of ray tracheids in newly formed rays, the thickness of the horizontal and end walls of ray parenchyma cells, the number of pits per crossing field, and the height/width ratio of ray cells viewed tangentially.

Introduction

Previous papers by the author in the series on wood structure of the Cupressaceae have dealt with the comparative anatomy of *Thuja occidentalis* L., *Juniperus* spp., and *Libocedrus decurrens* Torr. (Bannan (6, 7, 8)). The present report sets forth the results of investigations on the three North American species of *Chamaecyparis*, namely *C. thyoides* (L.) BSP., *C. Lawsoniana* (Murr.) Parl., and *C. nootkatensis* (Lamb.) Spach.

Material

Most of the wood samples were gathered by the author. Collections were as follows: *C. thyoides* from the coastal lowlands of Ocean Co., N.J., *C. Lawsoniana* from three sites near Port Orford, Sixes, and Riverton, Ore., and *C. nootkatensis* at Government Camp, Mt. Hood, Ore. and east of Chinook Pass, near Mt. Rainier, Wash. Supplementary material of *C. thyoides* was kindly supplied by Mr. Rufus H. Page of the Bladen Lakes State Forest, N.C., and of *C. nootkatensis* by Mr. A. C. Molnar from Grouse Mt. near Vancouver, B.C. The total number of wood samples studied was three hundred.

The types of specimen are listed in Figs. 1-7 where data on various structural features are presented. The term "stems mature trees" refers to the outer

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Contribution from the Department of Botany, University of Toronto, Toronto, Ont. Assistance was provided by a grant in aid of research furnished by the University of Toronto.

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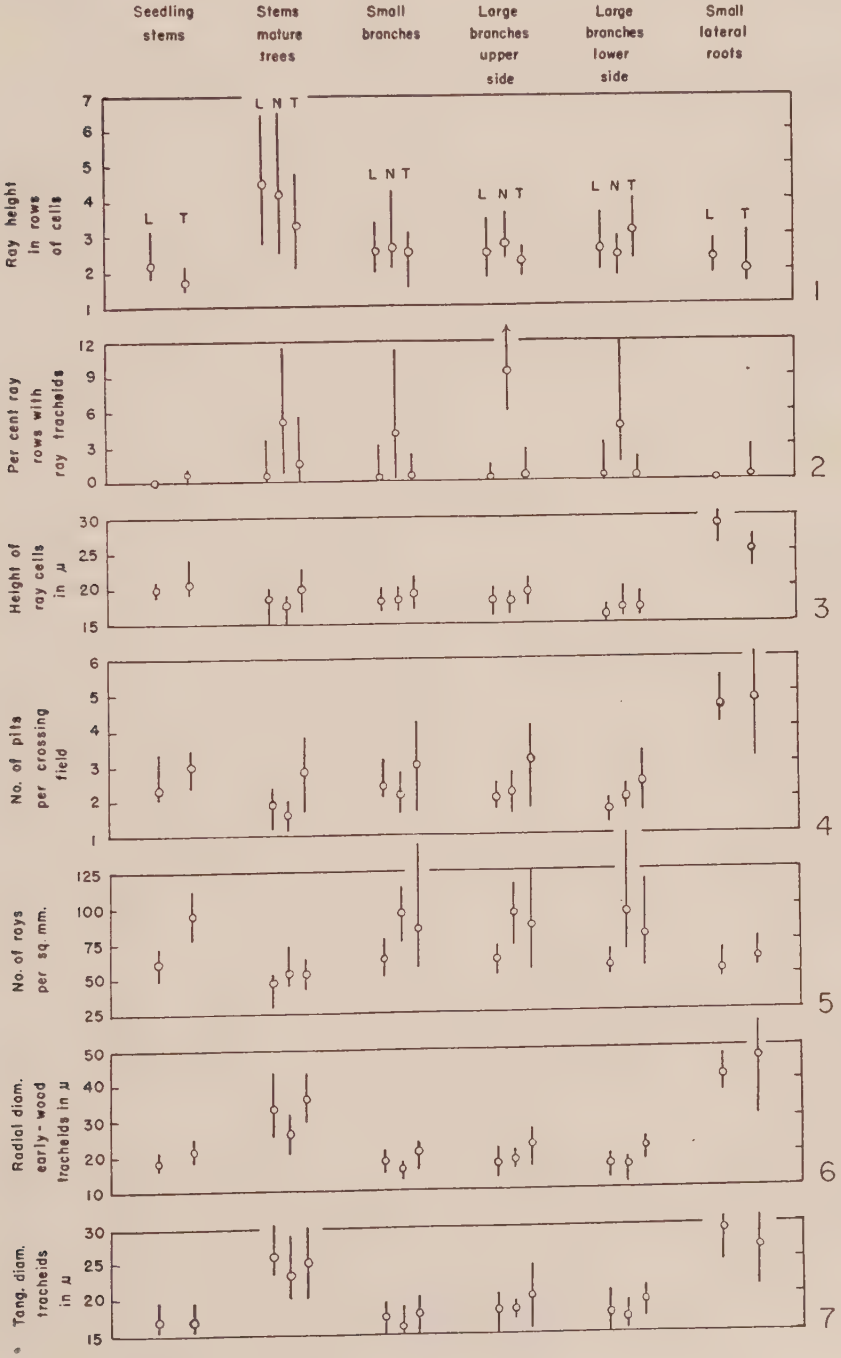
Figs. 1-7. Data on variability in the secondary xylem relating to cell size and frequency of occurrence of ray tracheids and rays. The vertical lines show the range from maximum to minimum means in different samples, and the small circle in each case indicates the grand average for all samples. The symbols *L*, *N*, and *T* denote *Chamaecyparis Lawsoniana*, *C. nootkatensis*, and *C. thyoides* respectively. The data are compiled from 300 samples. Fig. 1. Mean ray height in rows of cells. Fig. 2. Proportion of ray rows containing ray tracheids. Fig. 3. Mean height of cells in rays two or more rows high. Fig. 4. Number of pits per crossing field between early-wood tracheids and ray parenchyma cells. Fig. 5. Number of rays per sq. mm. of tangential section. Fig. 6. Radial diameter of early-wood tracheids in the growth rings. Fig. 7. Tangential diameter of tracheids as determined from transverse sections.

wood, usually about 1 meter above ground level, of trees 6 in. or more in diameter. The branch specimens were collected from mature trees, the term "small branches" referring to the inner wood 3 mm. from the pith and "large



branches" to measurements made at distances of 1-1.5 cm. from the pith. In some cases samples were taken from both distal and proximal portions of the branches. The roots examined were of the lateral type, growing more or less horizontally in the top few inches of soil. Wood samples were cut usually at distances of 2-3 meters from the stem, where the roots were 1-3 cm. in diameter. Measurements of the secondary xylem elements were made at a distance of 3 mm. outward from the primary xylem so as to be comparable with data for seedling stems and small branches. In Figs. 1-7 the upper and lower terminations of the vertical lines signify the maximum and minimum means discovered in the various wood samples studied, and the small circle breaking each line denotes the grand average for all wood samples in each category. In most cases only one or a small number of wood samples of stem, branch, and root wood were collected from each tree. Hence the data recorded in Figs. 1-7 are to be regarded as showing simply the variation among samples and not necessarily the differences between trees. Determination of the latter would obviously require examination of very many wood samples from the homologous parts of the trees being compared.

Tracheid Diameter

Fluctuations in tracheid diameter throughout the tree follow the trend common to conifers generally (Bailey and Faull (3), Bannan (6, 7, 8), Gerry (16), Harlow (18), and Sanio (26)). Tangential diameter increases outward from the center and is greater in lateral roots than in stems or branches of like size (Fig. 7). In the case of *C. nootkatensis* comparison of trees of similar size in different localities, namely Mt. Hood, Ore., Chinook Pass near Mt. Rainier, Wash., and Grouse Mt. near Vancouver, B.C., revealed only minor differences in tracheid diameter. As Myer (21) discovered in eastern white pine and hemlock, the intraregional variation much exceeded the slight interregional differences. Among the species of *Chamaecyparis*, *C. nootkatensis* generally has slightly smaller tracheids throughout all parts of the tree than *C. Lawsoniana* or *C. thyoides* (Figs. 6 and 7, and Table I).

The data on tangential diameter were obtained by measuring the width of 200 or more tracheids as seen in transverse section. While this is the usual method of calculating tangential diameter because of convenience, it does not reveal the true width of the cells. Checks on the maximum width of cells in tangential sections yielded mean values 27-30% above those given in Table I.

The radial dimensions of tracheids vary in different parts of the tree in much the same manner as tangential width. Radial diameter of the early-wood tracheids in the growth rings is greatest in lateral roots and least in the inner wood of branches and seedling stems (Fig. 6).

The shape of early-wood tracheids, as viewed in transverse sections, ranges from more or less squarish, pentagonal, or hexagonal in most radial files to narrowly rectangular in others. Transition to rounded outline becomes

TABLE I

DATA ON MEAN SIZE OR FREQUENCY OF STRUCTURAL FEATURES OF THE SECONDARY XYLEM OF *Chamaecyparis*

Part of tree	Species	Ray height in cells	Per cent ray rows with ray trach.	Height ray cells, μ	Tang. width ray cells, μ	Ratio height/ width ray cells	No. pits per cross field	No. rays per sq. mm.	Rad. diam. early-wood trach., μ	Tang. diam. trach., μ	Ratio - rad./tang. diam. trach.	Width annual rings mm.
Stems mature trees	*C. Laws.*	4.53	0.6	18.5	13.5	1.37/1	1.87	48	32.9	26.1	1.25/1	2.5
	C. noot.	4.06	5.0	17.2	14.1	1.22/1	1.61	55	26.8	22.9	1.17/1	0.9
	C. thy.	3.27	1.5	19.8	10.4	1.90/1	2.88	53	36.2	25.4	1.42/1	1.7
Upper side large branches	*C. Laws.*	2.50	0.4	17.8	11.5	1.55/1	2.03	64	17.8	18.0	0.99/1	0.4
	C. noot.	2.78	9.7	17.7	11.5	1.54/1	2.15	93	18.7	18.0	1.04/1	0.1
	C. thy.	2.38	0.4	19.0	10.7	1.78/1	3.11	86	23.1	20.2	1.14/1	0.4
Lower side large branches	*C. Laws.*	2.63	0.6	16.1	12.3	1.31/1	1.71	58	16.5	17.4	0.95/1	0.9
	C. noot.	2.46	4.7	17.1	11.5	1.48/1	1.96	93	16.7	17.3	0.97/1	0.2
	C. thy.	3.10	0.2	17.0	11.0	1.54/1	2.46	78	21.4	18.8	1.14/1	0.5
Small branches	*C. Laws.*	2.55	0.4	18.1	10.6	1.71/1	2.42	64	18.4	17.4	1.06/1	0.8
	C. noot.	2.65	4.1	18.2	10.5	1.73/1	2.21	95	16.7	15.7	1.06/1	0.2
	C. thy.	2.63	0.3	18.7	9.5	1.97/1	3.00	84	20.9	18.0	1.16/1	1.0
Seedling stems	*C. Laws.*	2.21	0	20.1	12.3	1.64/1	2.40	62	19.0	16.9	1.12/1	1.6
	C. thy.	1.78	0.7	21.0	11.9	1.77/1	3.02	92	20.7	17.1	1.21/1	1.8
Small roots	*C. Laws.*	2.32	0	29.2	14.9	1.96/1	4.6	52	41.0	29.4	1.40/1	——
	C. thy.	1.97	0.6	25.1	13.0	1.94/1	4.8	63	46.3	26.6	1.74/1	——

apparent in the "compression" wood of branches. The ratio of radial/tangential diameters of early-wood tracheids is 10-20% higher in *C. thyoides* than in other species (Table I). Intercellular spaces are easily discernible only in "compression" wood.

Tracheid Pitting

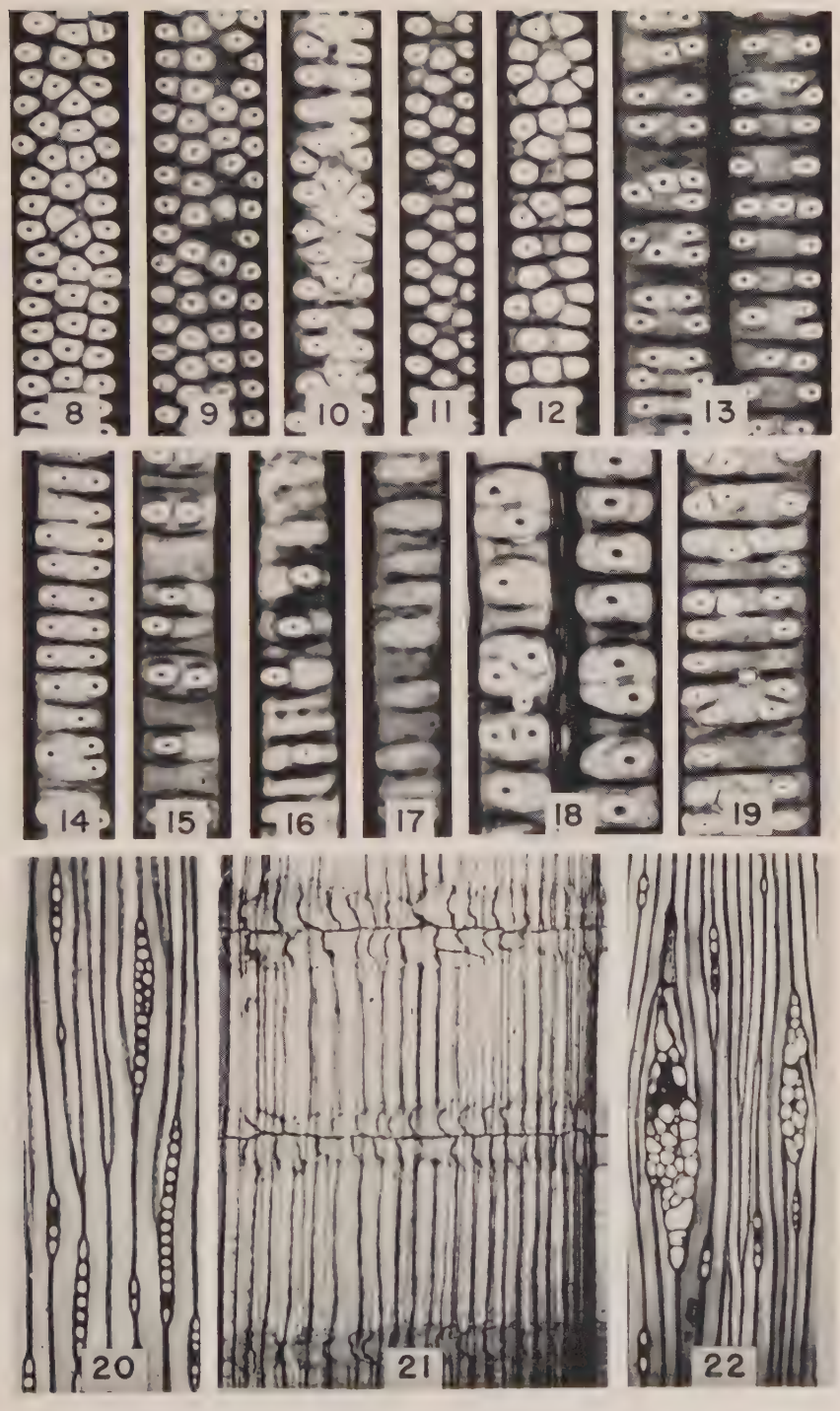
Radial intertracheary bordered pits are largest in roots where, in the early wood of the growth rings, their diameter averages approximately 15 μ. Size is slightly smaller in outer stem wood at 13-15 μ, and in branches the usual diameter is 9-11 μ.

The arrangement of the intertracheary pits is varied. In wood with narrow tracheids, such as in branches and young stems, the pits are typically uniseriate. In the outer rings of mature stems, where the xylem elements have greater diameter, the radial pitting in early-wood tracheids is usually uniseriate (Fig. 26) or less often biseriate, arrangement of pits in the latter instance being opposite or clustered. In the wood of small lateral roots 1-2 cm. in diameter, where the cells are widest, pitting in early-wood tracheids is usually biseriate (Figs. 14, 23), occasionally triseriate (Figs. 8, 11), and rarely quadriseriate (Fig. 9). Arrangement, while usually opposite or subopposite (Figs. 12, 13, 14, 15, 23), ranges to clustered (Figs. 18, 25) and alternate (Figs. 8, 9, 10, 11).

The pits are generally most frequent toward the tips of tracheids where in roots they are often crowded and alternate (Figs. 8, 9, 10, 11). Along the median portions of tracheids the pits are more widely spaced and the arrangement more regularly opposite (Figs. 14, 15). Considerable lengths sometimes lack pits (Figs. 16, 17, 24), the latter failing to develop on the primordial pit areas. The crassulae also fluctuate considerably in occurrence and in prominence. They tend to be most conspicuous along the central parts of the tracheids where the pits, when present, are typically opposite (Figs. 14, 15). Toward the ends of tracheids the occurrence of crassulae is less regular. Sometimes they are prominent and occur close to the tips of the cells, while in other cases they are faint or not apparent.

The controversy over the mechanism of cell elongation in the cambium has drawn attention to the comparative distribution of pits on the walls of consecutive tracheids. Priestley (25) argued that elongation of fusiform initials did not involve sliding growth at the tips but rather that extension occurred gradually over considerable lengths without slip between adjoining walls. As one item of evidence in support of his theory, he stated that a gradual shift in vertical position of the pits could be observed in radially successive tracheids.

FIGS. 8-18. *Chamaecyparis thyoides*, arrangement of pits in radial walls of early-wood tracheids in lateral roots. FIG. 19. *C. nootkatensis*, radial pitting in mature stem wood. FIG. 20. *C. nootkatensis*, tangential view of wood in old stem showing sporadic doubling of ray rows. FIG. 21. *C. nootkatensis*, radial of old stem showing recently formed rays composed exclusively of ray tracheids. FIG. 22. *C. Lawsoniana*, tangential of seedling stem showing abnormal rays. FIGS. 8-17, 19. ×250. FIG. 18. ×310. FIGS. 20-22. ×100.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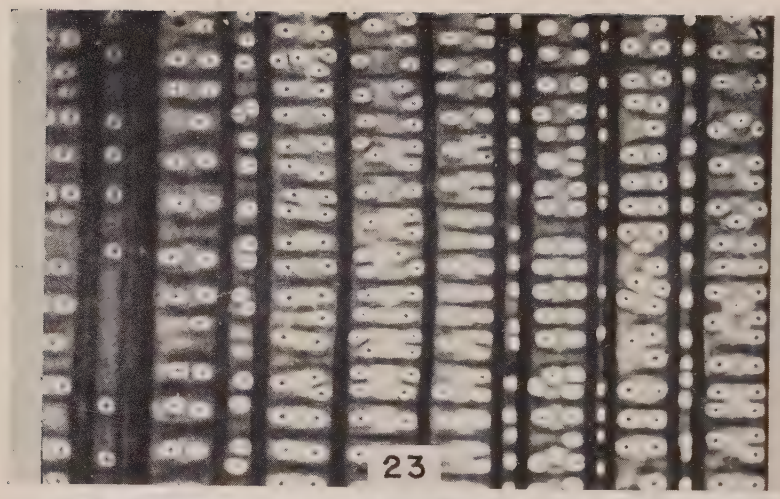
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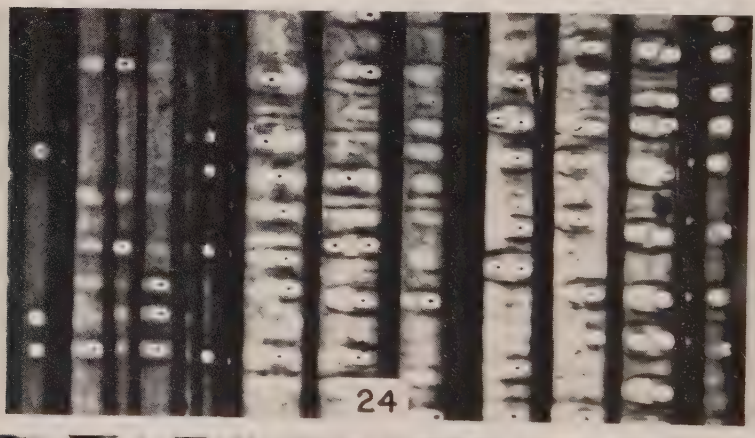
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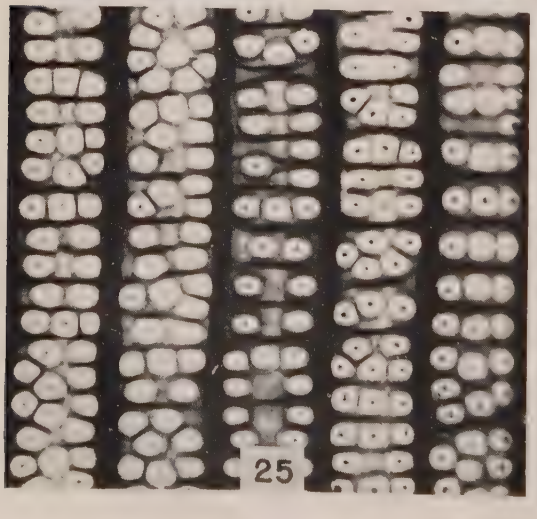
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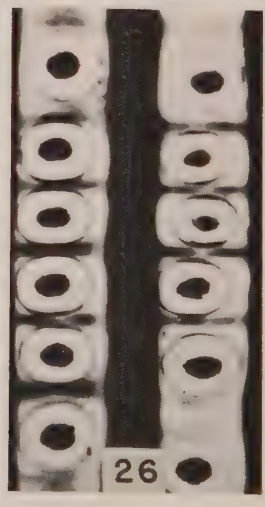
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The writer has been unable to confirm the existence of such a phenomenon. Actually various conditions obtain. Sometimes, particularly along the central parts of tracheids, the sites of primordial pits are in good radial alignment across successive cells, minor deviations occurring sporadically (Fig. 24). In other instances there is much alteration in size and position of the primordial pit areas in consecutive tracheids, the fluctuations not conforming to an obvious pattern. The expansion, contraction, and changes in position in different directions on the part of the primordial pit areas occur not only toward the ends of cells where elongation is proceeding, but sometimes also down the sides of cells which are not lengthening, as for instance in cells derived from fusiform initials whose tips are jammed against a blocking ray retarding or preventing terminal extension. Fig. 23 illustrates the changing positions of pits and crassulae in consecutive tracheids through the growth of five years in a root of *C. thyoides*. When pits are numerous continuity in position and arrangement in successive cells tends usually to become less definite or may be lacking (Fig. 25). Sifton (28) in his Fig. 11 also provides a striking example of the latter condition.

From his studies of the cambium and derived elements Bailey (1) concluded "There is no evidence, however, to indicate that the 'primordial pits' are permanent structures whose position remains unaltered during the life of the cambial initials", and that "the size, number, and orientation of the primordial pits appear to vary,—particular areas disappearing and others being formed *de novo* during periodic movements and displacements of the intercellular substance". Later Bailey (2) stated "the distribution of pits in secondary walls frequently bears no relation to the pattern of protoplasmic threads in primary walls". In this connection Figs. 18 and 19 illustrate an interesting phenomenon. Here, in a root of *C. thyoides* and a stem of *C. nootkatensis*, are shown bordered pits interrupting crassulae, thus seeming to arise between contiguous pit areas. Various lines of evidence indicate that cambial cell walls are readily adaptable to changing cell contacts, and that site of pit formation in the derived tracheids is not unalterably fixed by wall structure of the initiating cambial cells.

Tori usually show up distinctly in the radial intertracheary pits. On the whole they are rather better developed in stem and branch wood than in roots, and seem to be slightly larger in *C. nootkatensis* than in the other two species. Fig. 26 shows the torus in stem wood of *C. nootkatensis*. Here the torus extends slightly beyond the pit aperture and has a finely erose margin.

The crossing-field pits to ray parenchyma cells exhibit much diversity in number, size, and structure. The number per crossing field is especially varied. Some crossing fields are devoid of pits (Fig. 28), whereas others have as many as eight (Fig. 36). In general the number of pits per field decreases

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FIGS. 23-25. *Chamaecyparis thyoides*, arrangement of radial intertracheary pits in wood of lateral roots.

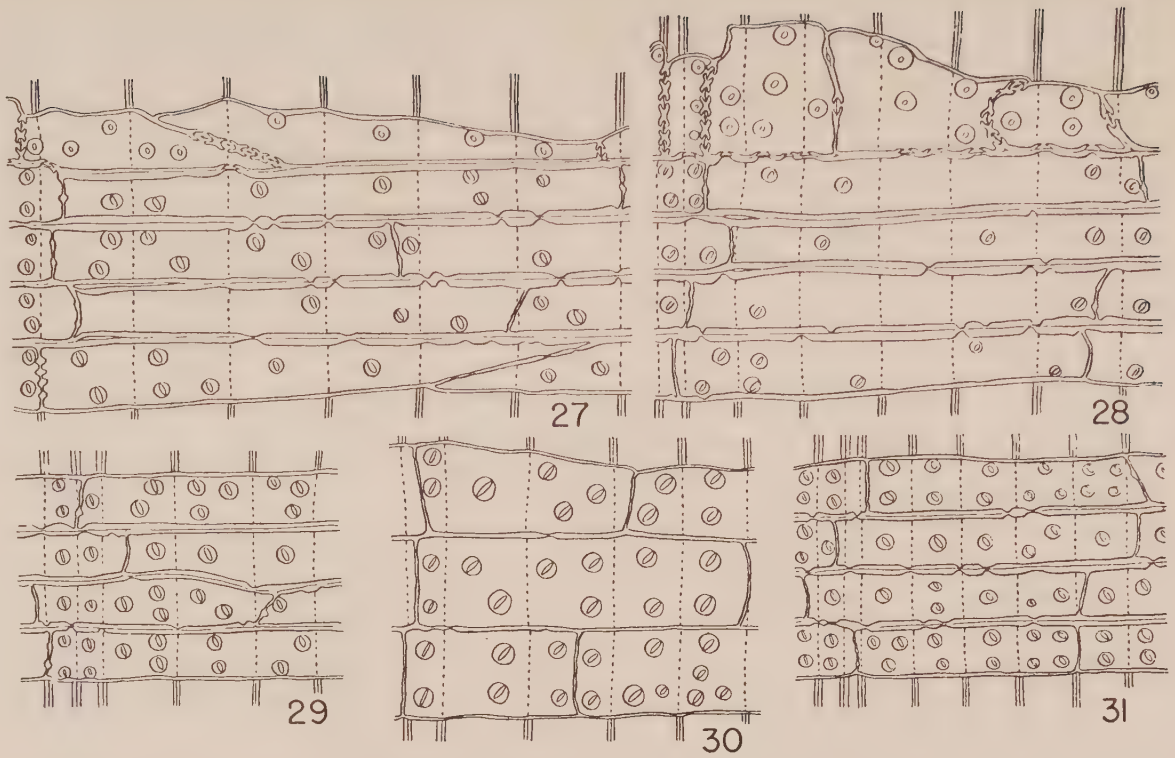
FIG. 26. *C. nootkatensis*, radial of old stem wood showing size and aspect of torus. FIGS. 23-24. ×185. FIG. 25. ×250. FIG. 26. ×630.

from the marginal rows of rays to the central rows (Figs. 32, 34, 36) and from the early to the late wood in the growth rings. However, noteworthy differences sometimes occur between the first and later formed parts of the early wood, and from one annual ring to the next. For example, counts made from 15,700 crossing fields in one sample of mature stem wood of *C. nootkatensis* revealed the mean number of pits per field in the first three tracheids of the early wood to vary from 1.18 to 1.73 in different annual rings, the mean for some rings diverging by as much as 30% from the grand average. In wide annual rings particularly the number of pits per crossing field tends to be less in the first few tracheids than in the subsequently formed part of the early wood. Cell adjustments consequent upon the initial surge of growth might perhaps be a factor in this relative sparsity of pits in the first-formed early wood. The fact that in the several old stems examined the mean number of pits per field was 10-20% less in the early wood of wide rings as compared with narrow rings in the same trees is probably not without significance in this connection.

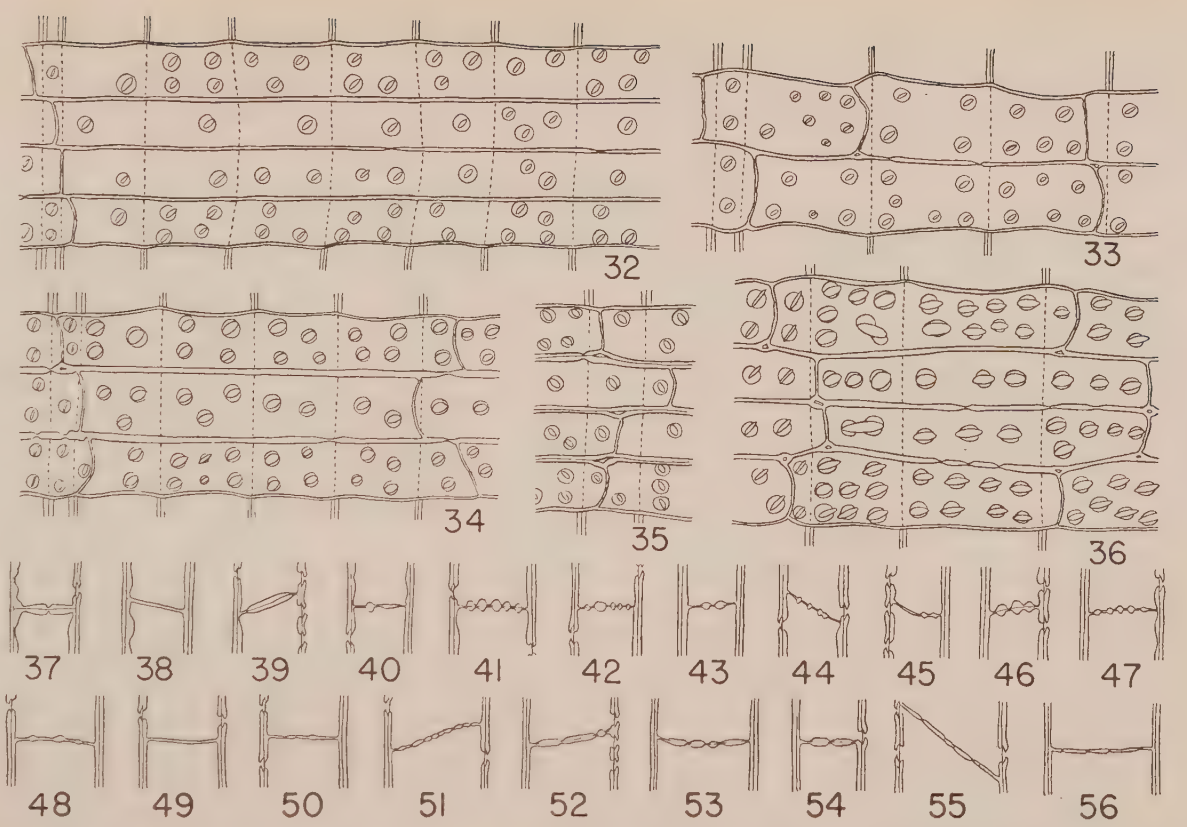
Comparisons of the crossing-field pits in different parts of the tree reveal these to be more numerous per field in roots than in stems or branches (Fig. 4). The means for homologous parts of the tree are appreciably higher in *C. thyoides* than in other species, but as in most other characters the specific ranges overlap broadly.

Diameter of the crossing-field pits fluctuates considerably, both in the same field and from one part of the tree to another. Usually the largest pits are found in roots (Figs. 30, 33, 36) and the smallest in the inner wood of branches and stems. No significant differences in mean size occur among the three species studied.

As regards structure, the crossing-field pits between tracheids and ray parenchyma cells are usually of the type described as "cupressoid". This type occurs throughout the Cupressaceae generally with the exception of *Thuja*. In cupressoid pitting the aperture in the tracheid wall is included, that is it does not extend beyond the pit border, and further, the aperture is usually narrower than the border. Such pitting is general throughout all parts of the tree in *C. nootkatensis* and in *C. Lawsoniana* (Figs. 27-33). In *C. thyoides* the apertures tend to be perceptibly wider (Figs. 34-36) and in roots especially the pit borders in the tracheid walls are sometimes relatively narrow (Fig. 36). As shown in the first and third ray rows from the top in Fig. 36 adjoining pits are occasionally confluent. Bailey and Faull (3) have noted such fusion of pit apertures, or of both apertures and chambers, in *Sequoia sempervirens*. There is also further deviation from the typical "cupressoid" type in that the pit apertures frequently extend beyond the border, particularly in roots (Fig. 36). Thus it is apparent that the crossing-field pits in *Chamaecyparis* range broadly in structure from "podocarpoid" through "cupressoid" and "taxodioid" types to a condition approaching the "window" pit or eipore (Gothan (17)). While these terms have descriptive value it must be recognized that much gradation in structure occurs in many conifers. This is especially true in *C. thyoides*.



FIGS. 27-31. *Chamaecyparis nootkatensis*, drawings from radial sections showing wall structure of ray cells and aspect of crossing-field pits in adjoining tracheids. FIGS. 27-29. Old stems. FIG. 30. Lateral root. FIG. 31. Upper side of large branch.



FIGS. 32-56. Wall structure of ray and xylem parenchyma cells. FIG. 32. *C. Lawsoniana*, ray structure in old stem. FIG. 33. Same in lateral root. FIG. 34. *C. thyoides*, ray structure in old stem. FIG. 35. Same in seedling stem. FIG. 36. Same in lateral root. FIGS. 37-46. *C. nootkatensis*, drawings from tangential sections of the peripheral wood of old stems showing aspect of the transverse walls of xylem parenchyma cells. FIG. 47. Same in lateral root. FIGS. 48-54. *C. thyoides*, same in stem wood. FIGS. 55, 56. Same in lateral root.

Xylem Parenchyma

Longitudinal parenchyma cells are varied in their distribution and frequency of occurrence. In *C. thyoides* and *C. Lawsoniana* these cells are generally common in stem and branch wood, and somewhat less so in roots. Arrangement varies from diffuse to zonate. In the vicinity of injuries the number of cells is markedly increased, often with tendency to zonation, the latter sometimes associated with quasi false-ring development. On the whole the tendency toward zonation is probably slightly more marked in *C. thyoides* than in *C. Lawsoniana*. In *C. nootkatensis* xylem parenchyma cells tend to be less common. Sometimes considerable areas in old stems are devoid of these cells. When present the distribution is varied, ranging from diffuse to zonate. In branches parenchyma cells are usually more common, with tendency to zonation.

The transverse walls of xylem parenchyma cells range from thin and smooth to nodular with pronounced thickenings. Thickness sometimes varies considerably among septa in a single longitudinal series of cells, but in the main the transverse walls tend to be thinner in roots than elsewhere in the tree, and to have more prominent beadlike thickenings in *C. nootkatensis* than in *C. thyoides* or *C. Lawsoniana*. Figs. 37-46 illustrate the range observed in the outer wood of old stems in *C. nootkatensis*, and Fig. 47 shows the less pronounced nodular aspect in roots. Figs. 48-56 depict the generally thinner walls in *C. thyoides*.

Size and Distribution of Rays

It is well known that rays vary considerably in size in different parts of the tree and from one specimen to another. In 1883 Essner (13) reported on such aspects of ray distribution as the trends outward from the pith, upward in the stem, fluctuations among samples of the same species, and differences between genera. Since Essner's time several authors have investigated the problem of intraspecific variation, with varying degrees of thoroughness, both from the standpoint of differences within the tree or between specimens (Bailey and Faull (3), Bannan (4, 5), Berkley (11), Fischer (14), Jaccard (19), and Myer (20)), and from the standpoint of the influence of environmental factors (Geiger (15), Harlow (18), Myer (20, 21), and Shope (27)).

In general the trends in height and distribution of rays in different parts of the tree resemble those described for such other Cupressaceae as *Thuja*, *Juniperus*, and *Libocedrus* (Bannan (6, 7, 8)). The average height tends to increase outward from the center in stems, and is more or less similar in small stems, branches, and roots (Fig. 1). The greatest height and widest range occur in the outer rings of old stems. A comparison of species shows the mean to be higher in the two western forms than in *C. thyoides* (Table I).

In keeping with the general tendency for mean ray height to increase outward from the pith to a maximum attained apparently at diverse ages in different trees, the mean height in the outer rings of trees 13-24 in. D.B.H. exceeded that in trees 6-12 in. in diameter. This was true in both *C. Lawsoniana* and *C. nootkatensis*. However, there was much variation among

neighboring trees of similar size. For example, mean height in nearby trees of *C. nootkatensis* on Mt. Hood, both 9 in. D.B.H., was 2.54 cells in one tree and 4.16 cells in the other, a difference of 64%. Again in the case of two trees 10 in. in diameter mean ray heights were 3.64 and 5.24 cells. These determinations were made from single wood samples. Extent of variation at different points around the circumference is unknown. Limited observations indicated some fluctuation in different sectors, particularly in excentric stems.

Some relationship appears to exist between mean ray height and width of annual rings. For example, in *C. nootkatensis* average ray height in the outer rings of stems 6-12 in. D.B.H. was 20% greater in 14 trees growing on the slopes of Mt. Hood, Ore., than in nine trees on Grouse Mt. near Vancouver, B.C. Mean width of the annual rings at the former site was 60% greater than at Grouse Mt. In another comparison between five trees 13-30 in. D.B.H. east of Chinook Pass, Mt. Rainier, and 12 trees of similar size on Mt. Hood, growth rates were similar and no significant differences were noted in mean ray height. Similar relationship is indicated in branches. In random selections specimens with wider rings tend to possess rays of greater height.

Various aspects of the general problem of intra-arboreal variation, differences between trees, and the influence of environment have been investigated at different times, often with dissimilar or inconclusive results. In some cases too few trees were sampled. While growth conditions appear to influence certain anatomical features, such as mean ray height, information on the subject generally is too scanty to be conclusive. The whole complex problem of variation requires much more study than has been attempted heretofore.

With regard to ray height it must be recognized that values of mean height vary according to the mode of determination. Authors interested in computing ray volume have customarily done so from tangential sections for obvious reasons. However, it should be emphasized that while tangential sections are useful for acquiring information on the frequency of rays, data on ray height from this source may be misleading. Sometimes there is considerable variation in adjoining areas. For example, in a stem of *C. Lawsoniana* the average ray height, as determined from 10 low power fields comprising 70-80 rays in each case, ranged from 2.77 to 3.36 cells. Similar fluctuation was discovered in other cases studied. Obviously determinations from a small number of fields may, by chance, be considerably in error.

It is even more important to recognize that values for mean height, even when determined from several tangential fields so as to minimize possible errors, may yet be inaccurate. The degree of inaccuracy depends to a large extent on the size and structure of the rays. Rays only one cell high are easily overlooked in tangential sections. Rays of recent origin, especially those in which the cells in the first-formed parts are radially separated, sometimes with gaps of considerable extent, often do not register in tangential sections. In contrast, these rays are readily discernible in strictly radial sections. Hence determinations of mean ray height from radial and tangential sections sometimes differ markedly. In most of the cases studied the values

for mean height were higher when calculated from tangential sections than from radial sections. The amount of difference ranged from 2-16%, the disparity being greatest in those cases where the average height was low and the proportion of rays one cell high or of recently formed rays was greatest. For these reasons all the data on ray height in this and in previous papers on conifer anatomy were determined from radial sections, the latter cut so as to parallel the course of the rays.

As regards specific differences in mean ray height, it is apparent from the data on Table I that *C. thyoides* tends to have slightly lower rays than the other species. This seems to be general in most parts of the tree, a contrary trend being noted only in some branches. The differences are most marked in the outer rings of mature stems, but it should be noted that even here the ranges of intraspecific variation for the three species overlap widely (Fig. 1). Graphs showing the relative frequencies of rays of various heights in the outer wood of stems 6-12 in. D.B.H. appear in Fig. 57. Most common ray height

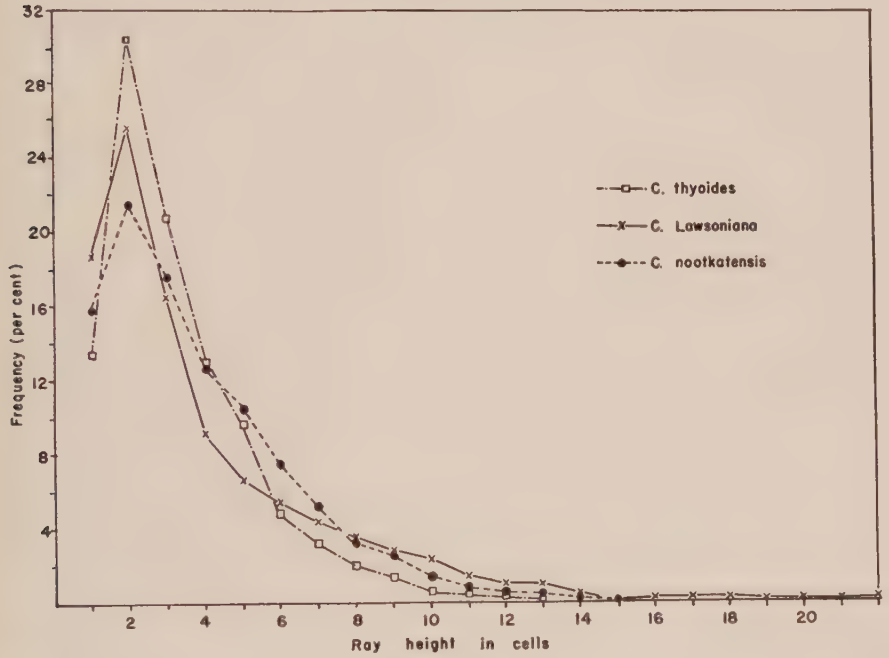


FIG. 57. Relative frequency of rays of different heights in the peripheral wood of stems 6-12 in. in diameter. Data based on 4900 rays with counts made from radial sections.

in all three species is two cells, but in *C. thyoides* the higher rays are less frequent than in western species. Absolute maximum heights discovered were 28 cells in *C. thyoides*, 33 cells in *C. Lawsoniana*, and 40 cells in *C. nootkatensis*. In stems of diameter greater than 12 in. the higher rays are more frequent than indicated in Fig. 57.

Nearly all the rays in North American *Chamaecyparis* are one cell wide, that is uniseriate. Occasionally some are partially biseriate (Fig. 20), but such

development is sporadic. Most frequent occurrence was in some old stems of *C. Lawsoniana* where the proportion of rays with one or more doubled rows ranged from 0.5 to 13%. Abnormal biseriate or multiseriate rays (Fig. 22) occur erratically. The structure and distribution of these have been dealt with in a previous article (Bannan (9)).

The frequency of rays fluctuates considerably among samples, but nevertheless some general trends are apparent in different parts of the tree. The number of rays per sq. mm. of tangential section tends to be highest near the pith of stems and branches and toward the center in roots, where the rays are low, and to decrease outward. Lowest frequency is in the outer rings of mature stems (Fig. 5) where the rays are higher and the intervening tracheids are wider than in the inner wood. No significant interspecific differences are apparent in old stems.

Ray Structure

Trends in the wall thickness of ray parenchyma cells in different parts of the tree are most evident in *C. nootkatensis* where the walls are thickest. In this species the horizontal walls of the ray cells are relatively thick in stem wood (Figs. 27-29) and in branches (Fig. 31), but are noticeably thinner in roots (Fig. 30). Comparison of similar material of the three species reveals the horizontal walls to be thickest in *C. nootkatensis* (Figs. 27-31) and thinnest in *C. Lawsoniana* (Figs. 32, 33), with *C. thyoides* more or less intermediate (Figs. 34-36).

In radial sections scattered pit-fields are readily discernible in the horizontal walls of the ray parenchyma cells in *C. nootkatensis* (Figs. 27-29), but are less obvious in the other species (Figs. 32-36), the walls often appearing unpitted. This apparent absence is due in part to the thinness of the walls and in part to the small size of the perforate areas. Examination of the walls in surface view, as seen in transverse sections, usually reveals small, scattered pit-fields of diverse sizes and shapes.

The end (tangential) walls of ray parenchyma cells are highly variable. In *C. nootkatensis* especially not only is there much variation among neighboring cells, but definite differences are sometimes detectable in the mean condition among samples from similar parts of different trees. Some walls are comparatively thin and appear to be of more or less uniform thickness from top to bottom. These walls may be described as "smooth", but when viewed in tangential sections are seen to be sievelike, the perforate area usually comprising most of the end wall. In other cases two or more sievelike areas occur. These are often separated from one another by wall thickenings which impart a nodular aspect to the end walls as seen in radial sections. Such thickenings range from small to pronounced. On the whole the nodular wall thickenings are best developed in stem and branch wood of *C. nootkatensis*, although it should be noted that much variation exists with a range from smooth to distinctly nodular (Figs. 27, 29). In roots, on the other hand, the walls are almost always more or less smooth (Fig. 30). In *C. thyoides* and *C. Lawsoniana* nodular walls are only sporadic. The beadlike thickenings are

less frequent and less well developed, the walls even in stem and branch wood generally appearing more or less smooth (Figs. 32, 34, 35).

In radial sections small intercellular spaces sometimes show up at the ends of ray parenchyma cells. These appear to be more common in *C. thyoides* (Figs. 34-36) than in the other species. In some instances the walls have the appearance of being slightly thickened adjoining the intercellular spaces. On the whole the ray cells are slightly narrower, tangentially, in *C. thyoides* than in *C. Lawsoniana* or *C. nootkatensis* which might possibly facilitate tangential extension and enlargement of the intercellular spaces usually present between the ray cells and adjoining tracheids. The latter spaces are clearly visible in tangential sections.

As is usual in conifers the size and shape of the ray parenchyma cells differ with position in the ray, location in the annual ring and part of the tree. Cells in the first-formed parts of new rays tend to be vertically extended or of varied irregular shapes, whereas in the succeeding parts they are customarily radially elongated. With regard to trends throughout the tree, it may be stated that mean height of the ray cells is greatest in roots and least in branches (Fig. 3). Actual means for the three species are 24-29 μ in roots, 17-20 μ in the outer wood of mature stems, and 16-19 μ in branches. These data were computed only from rays two cells or more high, thus excluding the irregularly shaped cells in new rays, generally composed of a single row of cells. The results indicate a slightly greater mean height of the cells in *C. thyoides* than in *C. Lawsoniana* or *C. nootkatensis* (Table I). Tangential width is likewise least in branches, greatest in roots, and apparently less in *C. thyoides* than in other species (Table I). In old stems the height/width ratio of ray cells, as viewed in tangential section, is 30-60% higher in *C. thyoides*, with reduced interspecific differences in other parts of the tree.

Ray tracheids do not occur in great numbers in *Chamaecyparis* but are generally more frequent in *C. nootkatensis* than in *C. thyoides* or *C. Lawsoniana*. These cells are habitually associated with the origin of rays, occurring in the first-formed parts of rays arising after varying amounts of secondary xylem have been produced. Such rays are usually one cell high, occasionally two rows high, or only rarely of greater height. Since the new-formed ray initials originate by segmentation of fusiform initials (Bannan (10)) the new rays tend to occur in vertical series, though owing to loss of varying proportions of the nascent ray initials only one or two rays may eventuate. As a rule the new rays stand apart from others, but when they happen to arise close by older rays they may become applied to the upper or lower margins of the latter. The proportion of tracheary cells in the first-formed parts of the new rays is variable, but is usually much higher in the outer wood of stems and branches than near the pith or in roots.

As mentioned above, some interspecific differences exist in the frequency of ray tracheids. In the outer wood of mature stems of *C. Lawsoniana* ray tracheids occur only sparsely in new rays. Most such rays are entirely parenchymatous, ray tracheids being present only in small numbers in a minority

of the new-formed rays. In *C. thyoides* the proportion of ray tracheids is somewhat higher. In contrast, in *C. nootkatensis* most new rays beginning in the peripheral wood of old stems are made up entirely or predominantly of ray tracheids. These new-formed rays, one or less often two rows in height, and composed largely or entirely of tracheary cells are a striking feature of *C. nootkatensis* (Fig. 21). Data from many specimens show specific differences in the mean frequency of ray tracheids to be of the following order. In the outer wood of stems exceeding 6 in. in diameter only 7% of the xylem rays one cell high contain ray tracheids in *C. Lawsoniana* as compared with 30% in *C. thyoides* and nearly 80% in *C. nootkatensis*. The actual differences in number of ray tracheids are greater than these values show since these cells occur sparsely along the new rays in *C. Lawsoniana*, usually being intermittent among the predominant parenchyma cells, whereas in *C. nootkatensis* most new rays are entirely tracheary. Expressed in terms of total ray tissue, that is including all rays, an average of only 0.6% of the ray rows in outer stem wood contain ray tracheids in *C. Lawsoniana* as compared with 1.5% in *C. thyoides* and 5.0% in *C. nootkatensis*. These data, however, should not be interpreted as indicating that the frequency of ray tracheids is an unfailingly reliable character for distinguishing the woods of American *Chamaecyparis*. Attention is directed to the fact that the values given above are the grand averages determined from all available samples of old stem wood. The variability among these samples, obtained from different trees, is very great. Actual ranges of variation, expressed in the same form as the mean values cited above, are 0-3.8% in *C. Lawsoniana*, 0-5.6% in *C. thyoides* and 0.8-11.4% in *C. nootkatensis* (Fig. 2). The existence of this broad fluctuation should be recognized when consideration is given to the use of ray tracheids as a diagnostic character.

The ray tracheids are of diverse shapes. In the earliest parts of new rays some are vertically extended, with successive later formed cells showing steps in the transition to the eventual radially elongated form characteristic of old rays. In *C. nootkatensis* the erect or upright form persists longer in the ray ontogeny than in other species, sometimes lasting through several years (Fig. 21).

As described elsewhere (Bannan (9)) ray tracheids also occur in abnormal uniseriate to multiseriate rays of sporadic occurrence in branches and small stems. In these rays the tracheary cells are interspersed among parenchyma cells, occurring in central as well as marginal rows, and are of varied sizes and shapes.

Discussion

It is clear that much variation exists in the size and to a lesser degree in the structure of the cells that comprise the secondary xylem. Differences, sometimes of considerable magnitude, occur not only among samples from similar parts of different trees but also in diverse parts of the same tree. In general

the latter type of variation is of broader range than the former. The intra-arboreal trends in *Chamaecyparis* resemble those described for *Thuja occidentalis*, *Juniperus virginiana*, and *Libocedrus decurrens* (Bannan (6, 7, 8)). This holds true for such anatomical features as tracheid diameter, size and distribution of radial intertracheary pits, size and number of pits in the crossing-fields between tracheids and ray parenchyma cells, the height and distribution of rays, cell size and wall characteristics of the ray parenchyma cells, and the occurrence of ray tracheids.

Certain of the anatomical characters listed above have been mentioned in the literature as useful both for the microscopic identification of *Chamaecyparis* wood and for specific differentiation within the genus. For instance, Penhallow (23), Brown and Panshin (12), and Phillips (24) considered the frequent occurrence of low, entirely tracheary rays to be a distinctive feature of *C. nootkatensis*. The author agrees that the frequency of ray tracheids is probably one of the best diagnostic characters if applied to wood samples of the proper type and used with caution. Obviously such a feature has no diagnostic value when dealing with those parts of the tree which habitually have few ray tracheids, such as lateral roots. Even in attempted identifications of samples from the periphery of old stems or large branches, in which wood ray tracheids are usually most numerous, usefulness of this feature is limited by the very great fluctuation in different samples. The three intraspecific ranges overlap widely.

Comparisons of the grand averages determined from homologous parts of many trees show slight interspecific differences in the mean height of rays, height and tangential width of ray cells, and the number of pits per crossing field. However, since the intraspecific ranges of variation overlap, these characters have diagnostic value only when used along with other features.

As regards such qualitative characters as wall thickness and pitting of ray and xylem parenchyma cells, it should be noted that these also vary. Peirce (22) described the end walls of ray parenchyma cells as smooth, whereas Phillips (24) cites *C. nootkatensis* as having nodular end walls, other species possessing smooth walls. The writer's observations support Phillips though it should be emphasized that the walls in *C. nootkatensis* are variable.

The author has not discovered in this study any single anatomical feature that is completely reliable for distinguishing microscopically the woods of North American species of *Chamaecyparis*. However, certain of the xylem features mentioned above, although variable, have diagnostic value when used together. In this category are the proportion of ray tracheids in the first-formed parts of new rays, the thickness of the horizontal walls and aspect of the end walls of ray parenchyma cells, the number of pits per crossing field between early-wood tracheids and ray parenchyma cells, and the height/width ratio of ray cells as viewed tangentially. The diagnostic worth of these features varies with part of the tree. Their value is greatest when applied to the peripheral wood of old stems or large branches. Some are of no use for specific differentiation of root wood.

The determination of the range of intraspecific variation and the true extent of specific difference requires careful study of very extensive material. This involves examination not only of samples from such parts of the tree as the roots, stems, and branches, but also from various sectors in the stem, different heights above the ground, and branches at various levels. Needed also are comparisons of neighboring trees in the same stand and of trees from diverse habitats and different localities. When dealing with characteristics of quantitative nature, such as cell size and frequency, careful consideration should be given to the method of determination. Calculations made from tangential or radial sections may differ substantially.

The need of extensive collections of material if reliable diagnostic criteria for distinguishing species or related genera are to be established was clearly demonstrated several years ago by Bailey and Faull (3). Nevertheless botanical literature continues to carry reports on investigations undertaken with the professed object of discovering the extent of similarity or difference between related forms, yet based on very small and sometimes nonhomologous samplings of the species being compared.

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EFFECT OF GROWTH IN ACID MEDIA ON THE MORPHOLOGY, HYDROGEN-ION CONCENTRATION, VISCOSITY, AND PERMEABILITY OF WATER HYACINTH AND FROGBIT ROOT CELLS[1]

By Wm. Harold Minshall[2] and G. W. Scarth[3]

Abstract

Roots of water hyacinth (*Eichhornia crassipes* (Mart.) Solms.) and frogbit (*Hydrocharis morsus-ranae* L.) when grown in solution cultures adjusted to pH 3.5, 4.0, 5.0, 6.0, and 6.5 did not suffer decidedly adverse, direct effects from hydrogen ions except at extreme acidities represented by a pH value of less than 4. During cool weather the plants were able to grow at a somewhat higher concentration of hydrogen ions than was possible in the warm summer months. The boundary between good growth and toxicity was a narrow one. Extreme acidity, short of the critical point, inhibited the growth of the roots by decreasing cell division and cell elongation. In the roots investigated, cell division at pH 3.5 was reduced to one-half of the rate at pH 5.0 and this reduction accounted for three-quarters of the inhibition in the growth of the roots. When grown in a highly acid medium, root cells acquired a resistance to the hydrogen ions. Water hyacinth meristem cells from the highly acid media exhibited a pronounced resistance to hydrochloric acid. It is suggested that this increased resistance is due to the cells having acquired a greater impermeability to the acids. Determination of the pH of expressed root juices revealed that, although all of the saps were decidedly less acid than were the media in which the plants were grown, roots grown in an extremely acid medium had a slightly more acid sap than those grown in a less acid one. A comparison of rates of streaming and the action of protoplasts during plasmolysis indicated that extreme acidity in the growth medium increased the viscosity of the cytoplasm of root cells. No significant difference was noted in the osmotic pressure of the root cells from the different cultures. The absolute permeability of frogbit root hairs to thiourea, as calculated from the formula $P_s = \frac{d}{6t}$, did not vary significantly from .0012 millimoles per square centimeter of surface per hour per molar concentration difference for plants grown within the range of pH 3.6 to pH 6.0.

Introduction

During the past four decades, hydrogen ion effects have played a prominent though somewhat controversial role in investigations on the growth of plants in soil. Subsequent to the discrediting of the belief that acidity, *per se*, was harmful to plants, there followed a period in which the hydrogen-ion concentration was considered to be the prime factor controlling plant growth and plant distribution. Although later work did not support the ascribing of such a unique position to hydrogen-ion concentration, there is ample evidence to

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prove that it is one of the important factors exerting an influence on the growth of plants. Seifriz (22) has termed the hydrogen ion "the most important ion in physiological reactions".

That extreme acidity in the growth medium reduces the extent of the root systems of plants has been indicated by the work of several authors, including Arnon and Johnson (2), Howell (11), Minshall (19), Singh and Mitra (23), Weissman (28), and Wilson (30). They found that as the growth medium became less acid the roots became more numerous, increased in length, and possessed a greater number of fine lateral rootlets. In most cases, however, precise determinations were not made of the amount of inhibition of root growth due to the acid medium or of the manner in which the inhibition was brought about.

Barth (4) studied the effect of short-time exposures to various acids on the viscosity of the protoplasm of *Arbacia* eggs. He found that the viscosity remained constant until a low pH was reached; it then increased very rapidly over a short range of pH, and ended in irreversible coagulation of the protoplasm. Carbonic and acetic acids were more active than hydrochloric or sulphuric acids, the latter having no effect at hydrogen-ion concentrations at which the former coagulated the protoplasm. The pH necessary to produce coagulation varied with the length of time of exposure of the eggs to the acid —the shorter the period of exposure, the higher the concentration of acid required for coagulation. Although the work of Barth indicated that hydrogen-ion concentration affected the physical state of protoplasm, a search of the literature has failed to reveal any information on the effect of external pH on the viscosity of the protoplasm of plant cells.

The possibility that the external hydrogen-ion concentration might affect the permeability of cells was suggested by the work of Hitchcock (10). He found that the permeability of gelatin-coated collodion membranes, as measured by the flow of water or of dilute solutions through the membranes, varied with the pH of the solution. The permeability was greatest near the isoelectric point of the protein. With increasing concentration of either acid or alkali, the permeability decreased, passed through a minimum, and then increased. A hypothesis that acidity may alter the permeability of plant cells, resulting in injury, is mentioned by Maximov (16). The hypothesis is based on the assumption that, at the optimum pH for a given plant, the permeability of cell protoplasm to salts is at a minimum. With a shift in the pH to either side of this optimum, the permeability may increase too much, resulting in injury to the plant. No information has been found in the literature, however, on the effect of external acidity on the permeability of plant cells.

It is apparent from the above that most studies of the effect of acid media on plants have dealt with the immediate or direct action of the acid on the plant or its cells. Little is known of the manner in which plant cells grown for a period of time in a highly acid culture medium differ from those grown at a more normal hydrogen-ion concentration. No attempt has been made to determine whether there is physiological adaptation by the plant when

grown in an acid medium. A previous enquiry (19) dealt with the role of hydrogen ions in the control of weeds. The present investigations were undertaken to gain information on the effect of hydrogen-ion concentration on the physiology and morphology of root cells.

Materials and Methods

As the cells of plants most subject to variations in external hydrogen-ion concentration are those of roots and as the roots of floating hydrophytes are convenient for cellular investigations, water hyacinth (*Eichhornia crassipes* (Mart.) Solms.) and frogbit (*Hydrocharis morsus-ranae* L.) were selected for study.

Water hyacinth plants possess slender, unbranched, adventitious roots which, although they rarely develop root hairs, are clothed with innumerable small horizontal lateral rootlets. These laterals are identical in that they all attain approximately the same length. Throughout the experiment, the water hyacinth plants grew well, producing in the less acid solutions an average of two new leaves and one new offshoot per week. The offshoots were removed as they appeared and, in order to keep the plants in a vegetative state of growth, the flower stalks, which started to develop in April, were likewise removed. In addition, the old roots and necrotic leaves were cut off at weekly intervals. As new adventitious roots were produced continuously from the rhizome at the base of the leaf petioles, the same individual plants supplied material throughout the experiment. The water hyacinth plant grown at pH 3.5 was a vegetative offshoot from the plant at pH 4.0, but, in the other hydrogen-ion concentrations, different individual plants were used.

Frogbit plants have a number of greenish colored, unbranched roots. They arise from the very short stem and hang down into the water. Along the greater part of their length, these roots bear a large number of unusually long root hairs. Numerous lateral stolons, each of which produces a new plant, also arise from the short stem. The persistent appearance of new plants from stolons, together with the rapid growth of these plants, provided an excellent source of root material for experimental purposes. Periodically, while the culture solutions were being renewed, the old plants were discarded and only one or two small plants were left in each hydrogen-ion concentration. All of the frogbit plants employed in this investigation were propagated vegetatively from a single individual.

The plants were grown in the greenhouse in solution cultures from November to May, with the period of daylight lengthened by means of 300-watt incandescent lamps. Six-liter glass museum jars, enclosed in heavy paper jackets, were employed as containers for the water hyacinth plants; the frogbit plants were grown in 1-gal. glazed crocks. The basic nutrient was Shive's R_5C_2 solution at one-fifth of its normal concentration. Distilled water was employed throughout. Iron was supplied as iron citrate. The main constituents

of the nutrient solutions are given in Table I. A supply of trace elements was added to the solutions, giving the following concentrations in parts per million: B, 0.5; Mn, 0.5; Zn, 0.05; Cu, 0.02; and Mo, 0.01.

TABLE I

COMPOSITION OF NUTRIENT SOLUTION

Chemical	Gram-molecular conc.	Gm. per liter
$Ca(NO_3)_2$	0.0010	0.17
$MgSO_4$	0.0030	0.36
KH_2PO_4	0.0036	0.49
HCl	0.0005	0.018
$FeC_6H_5O_7.3H_2O$	0.00001	0.003

In order that some growth would be obtained, it was desired to maintain the most acid solution just above the toxic pH. During February, pH 3.3 permitted a small amount of growth in water hyacinth, but, as the temperature of the solution increased owing to an increase in solar radiation, it was found necessary to raise the pH of this solution. For March, April, and May, when the majority of the determinations reported in this paper were carried out, the most acid solution used with water hyacinth was maintained at pH 3.5. With frogbit, the most acid solution was also maintained at pH 3.5 for the major portion of this period, but, during the latter part of April, it was found necessary to raise this solution to pH 3.6.

The desired reactions of pH 3.5, 4.0, 5.0, 6.0, and 6.5 were obtained by adding 0.018 gm. hydrochloric acid to each liter of solution and then adjusting with 0.016, 0.088, 0.144, 0.260, and 0.440 gm. sodium hydroxide, respectively, per liter of solution. Although preliminary investigations were carried out on the plants growing at all five reactions, most of the determinations reported in this paper were made on the plants growing at those hydrogen-ion concentrations which produced obvious growth differences, viz., pH 3.5, pH 4.0, and pH 5.0.

An attempt was made to maintain the initial pH of the nutrient solutions with fluctuations not exceeding ± 0.1 pH. Trelease and Trelease (25) found that the hydrogen-ion concentration of the culture solution remained constant if a suitable balance was found between the NO_3 and NH_4 ions. Supplying some of the nitrogen as NH_4 in the form of NH_4NO_3, however, increased the fluctuations of pH with the water hyacinth plants. Even with NH_4/NO_3 present in the ratio of 1/60, the NH_4 was taken up in preference to NO_3, as determined by the change in solution pH. With water hyacinth, therefore, it was not practicable to maintain a constant pH by adjusting initial NO_3/NH_4 ratios. As a further aid in maintaining a constant pH, potassium acid phthalate was added as a buffer to the nutrient solution. The phthalate buffered the solutions well, but the growth of a bacterial scum on this organic constituent counteracted its effect. Growth of the organism with consequent

removal of phthalate, produced greater changes in pH than did the growth of the plants in the unbuffered solution.

Following the failure of the adjustment of NO_3/NH_4 ratios and of the addition of phthalate buffer to produce a stable pH of the nutrient solutions, the desired hydrogen-ion concentrations were maintained by frequent renewal of solutions together with the daily adjustment of their pH. Owing to the difficulties described elsewhere (18), frequent renewals were found essential for water hyacinth, otherwise the rapid absorption of certain salts after renewal, followed several days later by an exosmosis of salts, produced extremely large fluctuations in the hydrogen-ion concentration of the external solution. In order to overcome these fluctuations the solutions were renewed every five days. Between renewals, the pH was determined daily with a quinhydrone electrode and, if necessary, it was adjusted with either hydrochloric acid or sodium hydroxide. For water hyacinth, the average fluctuation from the desired reactions was 0.03, 0.11, and 0.21 pH for the solutions maintained at pH 3.5, 4.0, and 5.0, respectively. For frogbit, the average fluctuation from the desired reactions was 0.05, 0.06, 0.08, 0.04, and 0.06 pH for the solutions maintained at pH 3.5, 4.0, 5.0, 6.0, and 6.5, respectively.

As difficulty was experienced in maintaining a constant pH of the solutions containing large vigorously growing water hyacinth plants, a root-separation method was devised whereby single roots could be grown in separate side-containers. By means of a ring on a retort stand, the plant was held at the edge of the culture jar. Two roots were then taken over the top edge of the jar and each root introduced into an adjacent 4-liter museum jar containing nutrient solution. To prevent the drying out of the short portion of the root exposed to the air, a small piece of cotton batting was placed over this portion of the root and was allowed to dip into the nutrient solution. Roots isolated in this manner appeared to develop normally in all respects, and the arrangement subjected simultaneously the roots of the same plant to three different hydrogen-ion concentrations. As in the side-container the ratio of root to solution was very small and as the duration of exposure was limited, there was no appreciable variation of solution pH when roots were grown under these conditions.

Osmotic Pressure Determinations

All osmotic pressure determinations were made by plasmolytic methods, with calcium chloride or sucrose as the plasmolyte. The plant material was placed in a graded series of dilutions for a period of from 15 to 20 min. Sections were then mounted in a drop of the plasmolyte and observed under the microscope to determine the concentration at which one-half of the cells exhibited incipient plasmolysis. As the molarity of this solution was known, the osmotic pressure was obtained from tables of concentration and osmotic pressure.

With water hyacinth, all determinations were made on the cortical cells of the lateral roots in the region just behind the root cap. Root sections, each

of which contained from 5 to 10 intact lateral roots, were obtained by cutting across the diameter of the main root. Sucrose was employed as plasmolyte as the protoplasts rounded up more quickly with it than with calcium chloride. A graded series of fresh sucrose solutions to cover the required range by increments of 0.01 volume molar were prepared daily. Determinations were made in duplicate or in triplicate and the coefficient of variation lay between 2 and 4%. Special precautions were required in osmotic pressure determinations with water hyacinth. As described elsewhere (18), the osmotic pressure of the root cells decreased approximately one-half an atmosphere after the root was severed from the plant. At room temperature, this decrease occurred within from one to three hours after the root was removed from the plant, but, when once it had taken place, the osmotic pressure of the root cells remained constant. To ascertain the osmotic pressure of the roots as growing in the cultures, therefore, it was necessary to carry out the determination immediately the root sections were removed from the plant. The various hydrogen-ion concentrations employed during the experiment did not affect in any way this decrease in osmotic pressure of the water hyacinth root cells.

With frogbit, all determinations were made on the root hairs. A graded series of calcium chloride solutions to cover the required range by increments of 0.005 weight molar were prepared at the start of the experiment and kept in well stoppered flasks. Small quantities of these solutions were placed in preparation dishes in order to carry out the determinations. In contrast to the osmotic pressure of the water hyacinth root cells, that of the frogbit root hairs did not change following the severance of the root from the plant.

Permeability Determinations

The penetration of thiourea into the root cells was measured by means of the deplasmolysis time and osmotic value methods. Scarth (21) defended the validity of plasmolytic methods and gave suggestions for the technique of the deplasmolysis time method. The osmotic value time was determined by Levitt and Scarth's (14) modification of the method of Huber and Schmidt. Scarth recommended a twice isotonic penetrating solution for the determination of solute permeability by the deplasmolysis time method. When plasmolyzed to one-half their original volume, however, the majority of the cells of water hyacinth roots died just previous to becoming deplasmolyzed. They died regardless of the material used as plasmolyte or how slowly the deplasmolysis took place. It was apparent that the degree of plasmolysis was the cause of the injury. It was found possible to overcome this difficulty by using $1\frac{1}{3}$ isotonic solution as penetrant. As a further precaution, the penetrating solution was made up of equal parts of penetrant and sucrose, i.e., $\frac{2}{3}$ isotonic penetrant plus $\frac{2}{3}$ isotonic sucrose.

With frogbit all permeability determinations were made on the root hairs. As many of the root hairs in the most acid solutions were killed by even slight plasmolysis and as the root hairs in the least acid solutions were so long that it was impossible to observe the total cell length for the point of desplasmolysis,

the osmotic value method was employed for this species. The osmotic pressure of representative root hairs was first determined. Fresh root sections were then placed in $\frac{2}{3}$ isotonic thiourea. At intervals of 5 or 10 min. a section was removed, mounted in a drop of $\frac{2}{3}$ isotonic thiourea plus $\frac{2}{3}$ isotonic sucrose, and then observed under the microscope for plasmolysis. A record was made of the length of time of exposure to the thiourea required to cause the plasmolyzing mixture to produce plasmolysis in one-half of the cells, that is to say, the time required for the average osmotic value of the cell to rise to 4/3 of its original value. This time was designated the "osmotic value time". Even if the cells died during plasmolysis, it was possible to determine whether or not the root hairs plasmolyzed by observing them under the microscope immediately they were mounted in the mixture of penetrant and plasmolyte. Many roots exhibited a gradient in osmotic pressure from the proximal to the distal portions. To smooth out discrepancies in the permeability values due to this gradient, the following practice was adopted. A root was removed from the plant and divided into three equal portions. Osmotic pressure of the root hairs in the middle portion was determined in calcium chloride. Permeability determinations were then made on the two end portions and these values averaged to give the osmotic value time for the root.

As the root hairs were cylindrical in shape and their diameter was easily measured, it was possible to calculate their absolute or "protoplasmic" permeability (permeability per unit area of protoplast). The formula used was derived as follows:

$$\text{Solute permeability } (P_s) = \frac{\text{amount of substance taken up}}{\text{time} \times \text{average concentration difference} \times \text{cell surface}}.$$

(1) Amount of substance taken up = increase of concentration $\times$ cell volume.

(2) Average concentration difference:

External concentration of thiourea $\equiv \frac{2}{3}$ osmotic pressure (O).

Internal concentration rises from zero to $\frac{1}{3}$ O.

Average of initial and final internal concentration.

$$\frac{O+\frac{1}{3}O}{2} = \frac{1}{6}O.$$

Average concentration difference $= \frac{2}{3}O - \frac{1}{6}O = \frac{1}{2}O$.

(3) Increase of concentration:

When cells show incipient plasmolysis in $\frac{4}{3}$O, the total internal o.p. is also $\frac{4}{3}$O of which $\frac{3}{3}$O is due to sap solutes and the remaining $\frac{1}{3}$O to the penetrant.

Increase of concentration $= \frac{4}{3}O - \frac{3}{3}O = \frac{1}{3}O$.

(4) Cell volume and cell surface remain unchanged during the test and, when cells are long in proportion to their width, the

$$\frac{\text{volume}}{\text{surface}} = \frac{L\pi r^2}{L2\pi r} = \frac{r}{2} = \frac{\text{diam.}}{4} \text{ (approx.).}$$

$$P_s = \frac{\text{increase of concentration} \times \text{cell volume}}{\text{time} \times \text{average concentration difference} \times \text{cell surface}},$$

$$= \frac{\frac{1}{3}O \times d}{t \times \frac{1}{2}O \times 4},$$

$$= \frac{d}{6t}.$$

It was necessary to measure the diameter of the cells and the osmotic value time. The permeability was calculated in millimoles per cm^2. of surface per hour per molar concentration difference.

With water hyacinth, all determinations were made on the cortical cells of the lateral roots in the zone just behind the root pocket. These cells were trapezoidiform in cross section and elongated slightly radially so that it was impossible to calculate accurately their volume from measurements made from the exterior of the root. For this reason, absolute permeability was not calculated for this species. Their relative permeability would vary with time, however, because the cells on which the determinations were carried out were from the same zone in the lateral roots, were approximately the same size, and were always plasmolyzed to the same constant relation to full cell volume. As the water hyacinth root cells were very delicate, great care had to be exercised in making the permeability determinations.

Most of the determinations on water hyacinth were carried out with the deplasmolysis time method. The osmotic value method was used to determine whether the mechanical effect of plasmolysis or of deplasmolysis was affecting permeability. As, for both methods, the penetrant had the same concentration in relation to the osmotic pressure of the cell, the value for the osmotic value method should approximate that of the deplasmolysis time method if the protoplasts were not injured during the determinations. They are not, however, equal owing principally to the difference in surface area of the cell during the determinations. Because in the deplasmolysis time method (1) the surface area is smaller than in the osmotic value method and (2) the surface area is changing all the time during deplasmolysis, the deplasmolysis time value should be slightly less than the osmotic value. When the results from the two methods were compared, it was found that the deplasmolysis time for the penetration of thiourea into the cortical cells of water hyacinth lateral roots approximated the osmotic value time. It was evident, therefore, that the deplasmolysis tests were a measure of normal penetration of thiourea.

The decrease in osmotic pressure that occurred following the severance of the root complicated the determination of cell permeability for water hyacinth.

As described elsewhere (18), this decrease in osmotic pressure was due to an exosmosis of salts. The increase in the osmotic pressure of the protoplast due to the penetration of thiourea was counteracted by the exosmosis of salt and as a result the permeability values were increased. Fortunately it was possible to overcome this difficulty by determining the thiourea permeabilities before or after the decrease in osmotic pressure had taken place. In all permeability tests the concentration of thiourea was a constant function of the osmotic pressure of the cell at incipient plasmolysis. As a result the concentration gradient between the osmotic pressure inside of the cell and outside of the cell would not be the same after the decrease in osmotic pressure as it was prior to this decrease; and permeability values obtained before or after the decrease in osmotic pressure would not be strictly comparable. When compared, however, it was found that the deplasmolysis time results obtained prior to the decrease in osmotic pressure were approximately the same as those obtained after the decrease had taken place. It was apparent, therefore, that (1) the decrease in osmotic pressure did not affect the deplasmolysis time value and (2) the difference in concentration gradient was not large enough to affect to any great degree the deplasmolysis time value. For practicable purposes, the values obtained at either time were comparable.

Details of the permeability methods developed for water hyacinth are as follows:

Deplasmolysis time method. —The osmotic pressure of representative cortical cells was first determined in sucrose solutions. Fresh root sections were then placed in the penetrating solution of $\frac{2}{3}$ isotonic thiourea plus $\frac{2}{3}$ isotonic sucrose. Every 5 or 10 min. sections were mounted in a drop of the penetrant and observed under the microscope for plasmolysis. The length of time required for one-half of the cells to deplasmolyse was determined. This time was designated the "deplasmolysis time". For the tests made after the decrease in osmotic pressure, the root was removed from the plant and was allowed to stand in its nutrient solution for at least five hours. By this time the decrease in osmotic pressure had taken place and the permeability test was carried through as outlined above. For the tests made before the decrease in osmotic pressure had taken place the lower half of the root was first removed from the plant, the osmotic pressure of its cortical cells determined as quickly as possible, and the penetrating solution prepared. A second portion of the same root was then removed from the plant, sectioned directly into the penetrating solution, and the permeability test carried out immediately.

Osmotic value method.—The osmotic pressure of representative cells was determined in sucrose. Fresh sections were then placed in the penetrating solution of $\frac{2}{3}$ isotonic thiourea. At intervals of 5 or 10 min. sections were removed from the penetrant, mounted in the plasmolyzing solution of $\frac{2}{3}$ isotonic thiourea plus $\frac{2}{3}$ isotonic sucrose, and observed for plasmolysis. A record was made of the length of time the sections had to be exposed to the penetrating solution to give plasmolysis in one-half of the cells. This time was designated the "osmotic value time". For determinations of osmotic

value time made either before or after the decrease in osmotic pressure, the techniques were the same as those described under the deplasmolysis time method.

Results

Growth

Although during these investigations no specific attempt was made to determine the critical or toxic hydrogen-ion concentration, some information was obtained concerning the toxic point. Whenever the concentration of hydrogen ions became critical the roots stopped growing and any roots that were present, having been produced under more favorable circumstances, soon became soft and gelatinous. In the present experiment, both water hyacinth and frogbit grew well at pH 4.0 and, at certain seasons of the year, they grew fairly well at pH 3.5. In November, at the start of the experiment, the water hyacinth plant placed in the culture at pH 3.5 receded gradually and died. In February, however, when a plant that had been growing at pH 4.0 for three months was transferred to pH 3.5 it continued to grow very well. During April it was necessary to decrease slightly, from a pH of 3.5, the concentration of hydrogen ions to prevent both species of plants from dying, and it is expected that further decreases would have been necessary if the experiment had been extended into the summer months. Although the toxic point varied at different times of the year, depending on the temperature, the results obtained corroborated the findings of Arnon and Johnson (2), Guest and Chapman (9), and Truog (26), namely, that decidedly adverse, direct effects from hydrogen ions do not occur except at extreme acidity as represented by a pH value less than 4. The boundary between good growth and toxicity was a narrow one.

Large water hyacinth roots were produced in all three of the most acid solutions (Figs. 7-9), but from Table II it is evident that the pH of the solution in which the plant was growing had an effect on the size of the roots. The shortest main root was produced at pH 3.5, the longest at pH 4.0, and those at pH 5.0 were intermediate in length. Lateral roots grown at pH 3.5 and pH 4.0 were much shorter, slightly thicker, and had somewhat smaller cortical cells than those grown at pH 5.0. Representative roots were selected for the measurements in Table II but, as the ages of the roots were not known, these measurements do not necessarily give the effect of the solution pH on the rate of growth.

TABLE II

THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE SIZE OF WATER HYACINTH ROOTS

pH of nutrient solution	Length of main root, cm.	Av. length lateral root, mm.	Av. width lateral root, μ	Av. length cortical cell of lateral, μ	Av. width cortical cell of lateral, μ
3.5	12	9	129	59	28
4.0	19	8	124	65	31
5.0	14	14	122	63	39

The effect of external pH on the rate of growth of water hyacinth roots was obtained by measuring single roots of the same plant grown in side-containers at two different hydrogen-ion concentrations. Measurements made on those portions of the roots produced during the seven days of the test are given in Table III. At pH 3.5 the main root grew less than half as

TABLE III

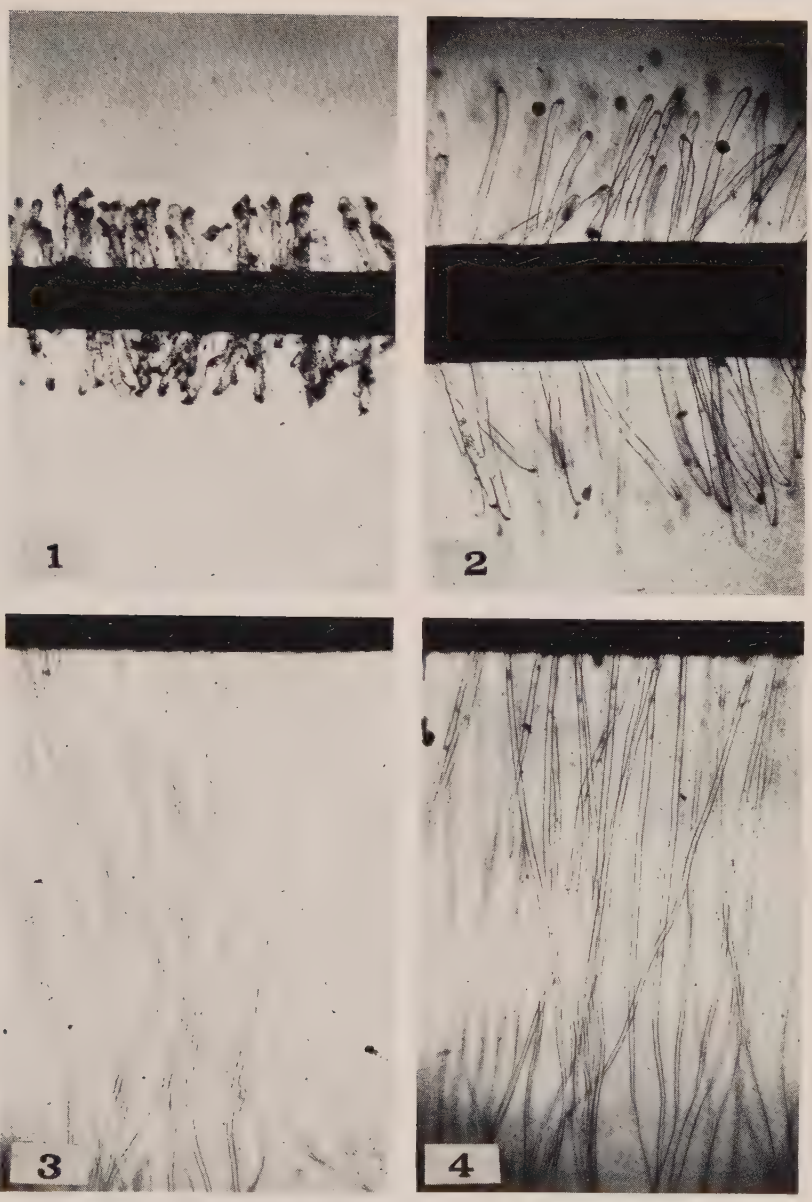
THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE DEVELOPMENT OF SINGLE WATER HYACINTH ROOTS WHEN GROWN FOR SEVEN DAYS IN SIDE-CONTAINERS

pH of nutrient solution	Length main root at start of test, cm.	Amount main root elongated, cm.	Length longest lateral root, mm.	Av. width lateral root, μ	Av. length cortical cell of lateral, μ	Av. width cortical cell of lateral, μ
3.5	8.0	3.7	6	110	49	27
5.0	9.5	7.7	14	135	66	40

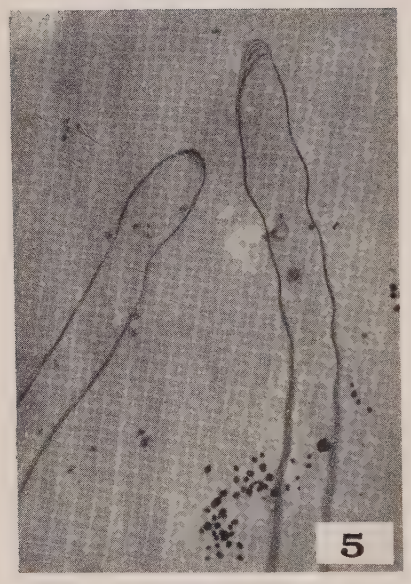
much as the one at pH 5.0. The laterals produced at pH 3.5 had smaller cortical cells, were slimmer and were less than half the length of those produced at pH 5.0. With the exception of the width of the laterals, the data obtained here were quite similar to those given in Table II for typical roots taken from the plants growing in the main containers at pH 3.5 and pH 5.0.

A study of cortical cell length in relation to length of lateral revealed that the difference in the length of the cells of the laterals grown at pH 3.5 and pH 5.0 was not nearly great enough to account for the total difference in the length of the laterals. From the measurements in Table III, it was calculated that approximately 25% of the difference in the length of the longest lateral of the roots grown at pH 3.5 and pH 5.0 was due to the difference in cortical cell length. Apparently the remaining difference was caused by an interference with normal cell division. When a count was made of the number of cortical cells in a longitudinal row from the proximal to the distal part of corresponding lateral roots, it was found that the lateral grown at pH 5.0 had almost twice as many cortical cells present as did the lateral grown at pH 3.5. Under the conditions of this experiment, therefore, the rate of cell division at pH 3.5 was reduced to approximately one-half of the rate at pH 5.0 and this reduction in rate was responsible for three-quarters of the difference in the length of the lateral roots.

One possible complication in this comparison of growth was that the tip of the main root might control the growth of the lateral roots and thus, if the growth of the main root were checked, it would indirectly affect the growth of the laterals. If, however, the tip of the main root did control the growth of the laterals, the removal of this tip should likewise influence the growth of the laterals. At various times during the experiment the tip was removed from water hyacinth roots. No effect was noticed on the laterals as a result of this treatment. They developed in a normal manner and their growth was



FIGS. 1-4. Effect of external hydrogen-ion concentration on the length of frogbit root hairs (×20). The black band running across each photograph is the main root. Fig. 1. pH 3.6. Fig. 2. pH 4.0. Fig. 3. pH 5.0. Fig. 4. pH 6.0.



FIGS. 5 and 6. Effect of external hydrogen-ion concentration on the diameter of frogbit root hairs (×80). Fig. 5. pH 4.0. Fig. 6. pH 5.0.

FIGS. 7-9. Effect of external hydrogen-ion concentration on water hyacinth roots (×1/6). Fig. 7. pH 3.5. Fig. 8. pH 4.0. Fig. 9. pH 5.0.

typical of the growth of roots with the tips attached. This result suggests that the checking of the growth of the main root in the acid solutions would not influence the growth of its laterals, and that the hydrogen-ion concentration would act independently on the root and on its laterals.

Average measurements of typical frogbit roots from the different hydrogen-ion concentrations are given in Table IV. The most acid solution (pH 3.6

TABLE IV

THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE SIZE OF FROGBIT ROOTS (AVERAGE VALUES)

pH of nutrient solution	Length of root, cm.	Length of root hair, μ	Diameter of root hair, μ
3.6	10	600	71
4.0	26	1500	73
5.0	27	5000	44
6.0	25	6000	45
6.5	25	6000	44

was the only solution that checked the length of the roots. With the more delicate root hairs, however, the two most acid solutions (pH 3.6 and pH 4.0) had a definite inhibiting effect on growth (Figs. 1-6). At pH 3.6 the root hairs were very short, but they were much thicker than those produced at pH 5.0. At pH 4.0 the root hairs were longer than those at pH 3.6, but not so long as those in the less acid solutions. They were approximately the same diameter as those produced at pH 3.6. Small knoblike protuberances were present on the walls of the root hairs grown at pH 3.6 and at pH 4.0 (Fig. 5). Lundegardh (15) found that, whenever growth was stopped by internal causes, similar protuberances developed on the root hairs of wheat. The root hairs in the other three solutions (pH 5.0, 6.0, and 6.5) were similar in size and all were well developed. If volumes are calculated from the measurements of the cells, the root hairs produced at pH 3.6 had approximately one-quarter of the volume of those produced at pH 5.0 or pH 6.0, but the root hairs at pH 4.0 had three-quarters as much volume. Throughout the time that the frogbit plants were growing in the solutions, there was little variation in the size of their roots or of their root hairs.

When roots of water hyacinth and frogbit from the various hydrogen-ion concentrations were killed and fixed in chromoacetic acid, washed, dehydrated in dioxan, embedded in paraffin, sectioned with a rotary microtome, stained with safranin and fast green, mounted in balsam, and examined under the microscope, no significant difference was found in the anatomy of the roots, other than that commensurate with root size.

Tolerance of Acidity

While studying the effect of various acids on the growth of *Lupinus albus* seedlings, Kahlenberg and True (13) observed that, when seedlings were left in the solutions that had killed the roots, lateral roots pushed out above the

dead region and grew in the solutions without serious harm. They suggested that a gradual increase in the concentration of the acid in the growth medium brought about an accommodation on the part of the plant, and, as a result, the plant tolerated concentrations of acid that previously would have proved fatal.

To compare the ability of the cells of the roots grown at different hydrogen-ion concentrations to endure exposure to acid, sections of roots were placed in flasks containing a series of hydrochloric or acetic acid of known normality and pH. At intervals, root sections were removed from the acids and tests were made to determine the number of root cells still alive. The tests used for this purpose were vital staining with neutral red; plasmolysis with calcium chloride or sucrose; and, in the case of frogbit, protoplasmic streaming. Preliminary investigations indicated that, although the degree of injury from exposure to the acid increased with an increase in the time of exposure, the trend of results was not affected by the length of time of exposure. For this reason it was not necessary to keep uniform the duration of exposure to the acids in the different tests.

Representative results from the 10 tests made with excised roots are given in Tables V, VI, and VII. It is evident that the root cells grown in the most

TABLE V

THE EFFECT OF GROWTH AT DIFFERENT HYDROGEN-ION CONCENTRATIONS ON THE CAPACITY OF WATER HYACINTH LATERAL ROOTS TO TOLERATE HYDROCHLORIC ACID

Hydrochloric acid		Meristem cells from nutrient solution pH			Mature cells from nutrient solution pH		
N	pH	3.5	4.0	5.0	3.5	4.0	5.0
		Cells alive after 5 hr. exposure, %					
1/666	2.8	100	100	0	0	0	0
1/2000	3.3	100	100	0	60	20	5
1/8000	4.0	100	100	10	100	100	95
1/28,000	4.6	100	100	10	100	100	100
Tap water	——	100	100	100	100	100	100
		Cells alive after 10 hr. exposure, %					
1/666	2.8	25	5	0	0	0	0
1/2000	3.3	50	30	0	5	0	0
1/8000	4.0	100	100	0	100	90	30
1/28,000	4.6	100	100	0	100	100	100
Tap water	——	100	100	100	100	100	100

acid medium (pH 3.5 or pH 3.6) were able to tolerate a higher concentration of hydrochloric or acetic acid for a given period of time than were the corresponding cells from the less acid solutions. In general, the greater the acidity of the culture medium, the more pronounced was the tolerance of the roots to both hydrochloric and acetic acids. With water hyacinth, the superior

TABLE VI

THE EFFECT OF GROWTH AT DIFFERENT HYDROGEN-ION CONCENTRATION ON THE CAPACITY OF WATER HYACINTH LATERAL ROOTS TO TOLERATE ACETIC ACID

Acetic acid		Cells alive after 4 hr. exposure, %					
		Meristem cells from nutrient solution pH			Mature cells from nutrient solution pH		
N	pH	3.5	4.0	5.0	3.5	4.0	5.0
1/500	3.6	0	0	0	0	0	0
1/1000	3.9	20	0	0	90	25	25
1/10,000	4.5	100	50	0	100	75	90
1/100,000	5.2	100	100	100	100	100	100
Tap water	——	100	100	100	100	100	100

TABLE VII

THE EFFECT OF GROWTH AT DIFFERENT HYDROGEN-ION CONCENTRATIONS ON THE CAPACITY OF FROGBIT ROOT HAIRS TO TOLERATE EXPOSURE TO HYDROCHLORIC ACID FOR 17 HR.

Hydrochloric acid		Percentage of cells alive from nutrient solution pH				
N	pH	3.6	4.0	5.0	6.0	6.5
1/2000	3.3	100	100	0	0	0
1/4000	3.6	100	100	10	10	0
1/8000	4.0	100	100	10	50	50
1/14,000	4.2	100	100	100	25	50
1/28,000	4.6	100	100	100	50	50
Tap water	——	100	100	100	100	100

toleration of hydrochloric acid was much more conspicuous for the meristem cells of roots grown at pH 3.5 and pH 4.0 than for the mature cells from the same media (Table V). Throughout these tests with excised roots, it was observed that, when water hyacinth laterals grown at pH 3.5 or pH 4.0 were placed in the series of hydrochloric acid, the meristem area was the last portion of the root to die, but, with laterals grown at pH 5.0, the meristem was always the first portion to die. To acetic acid, however, the meristem cells of the water hyacinth roots grown at pH 3.5, pH 4.0, or pH 5.0 did not exhibit so great a degree of tolerance as did the older cells from the same roots (Table VI).

Although from the results obtained it is not possible to estimate quantitatively the relative resistance of the cells to the acids, yet it is evident that (1) growth in an extremely acid medium increases the tolerance of root cells to both hydrochloric and acetic acids and that (2) the superior toleration of hydrochloric acid is much more conspicuous in the meristem cells grown at pH 3.5 or pH 4.0 than it is in mature cells from the same hydrogen-ion concentration or in either meristem or mature cells from less acid media. As the difference in toxicity between weak and strong acids of equal $[H^+]$ is largely due to their well known difference in penetrating power, a comparison of the

results with hydrochloric and acetic acids throws a certain amount of light on the mechanism of adaptation. The relative resistance to both the strong acid, hydrochloric, and the weak acid, acetic, increased with increasing acidity in the growth medium. It seems probable, therefore, that the increased resistance may be ascribed to the cells having acquired a greater impermeability to the acids. Likewise, the remarkable toleration to hydrochloric acid of the meristem cells grown in the more acid media may be ascribed to their having acquired even greater than normal impermeability to the strong acid. As meristem cells from all the culture media were less resistant to acetic acid, a ready penetrant, than were mature cells from corresponding media, it is evident that meristem cells are rather less tolerant of increased acidity within the cell. This conclusion supports the supposition that the acquired resistance of the meristem cells grown in the more acid media is largely a matter of permeability. A certain degree of increased tolerance to acetic acid, and presumably to internal acidity, is displayed by both meristem and mature cells as a result of acid growth conditions, but for some reason only the meristem cells acquire the much higher resistance to hydrochloric acid. That this great resistance to hydrochloric acid is confined to the meristem cells and not shared by mature cells is a puzzling phenomenon. The mature cells were formed in the same medium as were the meristem cells, a fact which indicates that they lost a part of the resistance which they once possessed.

pH of Root Cells

As yet the effect of the external hydrogen-ion concentration on the acidity of the cytoplasm or of the vacuole fluid of plant cells is not definitely known. Most of the results that are available give the effect of the pH of the external medium on the pH of the sap expressed from a composite sample of several tissues. Miller (17) and Hurd-Karrer (12) have reviewed some of the work in this field. In the present work it was not found possible to determine directly the internal pH of cells. Determinations were made, however, on the expressed saps from water hyacinth roots.

Water hyacinth roots from the three most acid cultures were rinsed in distilled water, dried on absorbent paper, and placed in bottles of convenient size. The bottles were corked and then immersed in boiling water for 20 min. After expressing the sap with a hand press, the pH of the sap was determined by means of a glass electrode.

From the results in Table VIII, it is apparent that the hydrogen-ion concentration of the growth medium had some effect on the pH of the expressed root sap. The acidity of the sap increased with increased acidity of the growth medium. The root sap, however, was considerably less acid than the solution in which the plant was grown, and the range of differences in the pH of the root saps was much smaller than the range of the corresponding media. These results agree with those reported by Arnon (1), Arnon and Johnson (2), Bryan (6), and Wadleigh, Robbins, and Beckenbach (27).

At various times during the experiment roots were exposed to the acid indicator dyes methyl red and ethyl red in an attempt to determine the pH

of the cells of roots grown at the different hydrogen-ion concentrations. Procedures tried included: exposure to water solutions of the dyes, exposure to sodium salt solutions of the dyes, adjustment of the pH of the dye solutions,

TABLE VIII

EFFECT OF EXTERNAL HYDROGEN-ION CONCENTRATION ON THE pH OF EXPRESSED SAP FROM WATER HYACINTH ROOTS

pH of nutrient solution	pH of expressed root sap
3.5	5.4
4.0	5.6
5.0	5.8

centrifuging the root material in the dye solution, and mounting of the roots in dilute acetic acid for observation. Failure resulted from all attempts at securing penetration of these dyes into the root cells in sufficient quantity to permit visual observation of color differences.

Viscosity of Cytoplasm

An indication of the effect of the hydrogen-ion concentration of the culture medium on the viscosity of cytoplasm was obtained for the root hairs of frogbit by measurement of rates of streaming under uniform conditions of light and temperature. The results given in Table IX indicate that the rate of stream-

TABLE IX

EFFECT OF EXTERNAL HYDROGEN-ION CONCENTRATION ON THE RATE OF STREAMING OF FROGBIT ROOT HAIRS

pH of nutrient solution	Time required for particle to travel 100 μ by streaming, sec.
3.6	13
4.0	9
5.0	6
6.0	8

ing in root hairs from the most acid solution was much slower than the rate in root hairs from the other solutions, and that, in general, the slower the rate of streaming the more acid was the nutrient medium.

To obtain evidence as to whether or not the slow rate of streaming was, in part at least, caused by higher viscosity of the cytoplasm, root hairs grown at pH 3.6 and pH 5.0 were placed in a series of calcium chloride solutions and observed under the microscope for protoplasmic streaming when plasmolyzed. The root hairs grown at pH 5.0 continued streaming in a 1.6-times isotonic solution; those grown at pH 3.6 ceased streaming in a 1.3-times isotonic solution. The protoplasts of the former were rounded up into several sections; those of

the latter formed a long rope-like mass in the center of the root hair. When water was added, this rope-like mass rounded up immediately but no streaming was observed and the rounded up portions soon burst and spilled out their contents. It was frequently observed in making osmotic pressure determinations on frogbit root hairs from the pH 3.6 solution, that many of the root hairs died even when plasmolyzed a slight amount. Adhesion of the protoplast to the cell wall would injure a plasmolyzing cell, but as the plasmolysis, when it did occur, was convex plasmolysis, adhesion did not appear to be a factor in the death of these cells. In contrast to this result with the root hairs grown at pH 3.6 was that obtained with those grown at pH 5.0. Unless plasmolyzed severely, the root hairs grown at pH 5.0 did not die during plasmolysis. All of the evidence obtained from the comparative rates of streaming and the action of the protoplasts during plasmolysis suggested that the growth of frogbit plants in an extremely acid medium increased the viscosity of the cytoplasm of their root hairs.

Osmotic Pressure

With roots as grown under the conditions of this experiment, the osmotic pressure of the cortical cells of water hyacinth laterals varied from 5.0 atm. to 5.5 atm., and that of frogbit root hairs varied from 5.0 atm. to 7.5 atm. As would be expected, the osmotic pressure of the root cells varied somewhat, the variation being greater in frogbit than in water hyacinth. The osmotic pressures of frogbit root hairs of the proximal portion of the root were from 1 to 2 atm. higher than those of the distal portion, and the root hairs from old roots exhibited a higher osmotic pressure than those from corresponding portions of young roots. As the osmotic pressure values obtained were within the limits of variation, the hydrogen-ion concentrations employed in this experiment had no significant effect on the osmotic pressure of root cells.

Permeability to Thiourea

The results in Table X indicate that variation of the hydrogen-ion concentration of the culture solution from pH 3.6 to pH 6.0 did not affect the protoplasmic permeability of frogbit root hairs to thiourea.

The permeability results with water hyacinth were not so consistent as were those obtained with frogbit. For the water hyacinth plants grown at pH 5.0, the normal deplasmolysis time of the cortical cells of the lateral roots was approximately 30 min. For the plants grown at pH 3.5, the deplasmolysis time of the cortical cells of the lateral roots was 35 min., but the difficulties encountered in determining permeabilities with this species preclude the consideration of such a difference as being significant. In contrast to these results, however, were the permeabilities of water hyacinth roots grown in side-containers. The cortical cells of the root grown in a side-container at pH 3.5 had a deplasmolysis time of 15 min. as compared to a deplasmolysis time of 25 min. for cells of approximately the same size from a similar root grown at pH 5.0. In all three experiments with roots in side-containers, the root grown in the most acid medium exhibited a faster penetration of thiourea than did

TABLE X

EFFECT OF EXTERNAL HYDROGEN-ION CONCENTRATION ON THE PERMEABILITY OF FROGBIT ROOT HAIRS TO THIOUREA

pH of nutrient solution	Diameter of root hair, μ	Osmotic value time for $\frac{2}{3}$ isotonic thiourea, min.	Protoplasmic permeability*
3.6	66	60	.0011
3.6	80	65	.0012
4.0	77	75	.0010
4.0	73	60	.0012
5.0	45	40	.0011
5.0	44	35	.0013
6.0	42	35	.0012
6.0	43	30	.0014

** Permeability in millimoles per square centimeter of surface per hour per molar concentration difference as calculated from the formula* $P_s = \frac{d}{6t}$.

the root grown in the least acid solution. No satisfactory explanation can be offered to account for the fact that, when single roots were grown in side-containers, an increased hydrogen-ion concentration in the growth medium increased the penetration of thiourea, but that, when entire plants were grown in the cultures, an increased hydrogen-ion concentration in the growth medium either had no significant effect or gave indications of a slight decrease in the penetration of thiourea. If this difference was a valid one, it may have been caused by the special treatment of growing roots in side-containers, or it may have been associated with the decrease in osmotic pressure encountered with this species. Although variable results were obtained with water hyacinth, no evidence was secured to suggest that, if the entire root system was growing in the solution, an increase in the acidity of the growth medium from pH 5.0 to pH 3.5 would increase the permeability of water hyacinth root cells to thiourea.

Discussion

The stiffening of the cytoplasm of the frogbit root hairs as a result of growth in a highly acid medium suggests that incipient acid coagulation may precede death and that coagulation may be the cause of death in an extremely acid growth medium.

Although an abnormally acid medium, like any other unfavorable environment, might be expected to reduce the growth rate by obscure and complicated interactions, the effect of acid on the type of growth suggests a simple hypothesis to explain the effects of "non-toxic" acidities. The decrease in length and the increase in thickness of the roots and root hairs in acid media is similar to the effect that Thimann (24) describes for an abnormally high supply of auxin. Inasmuch as the activity of auxin is increased by an increase of acidity in the external medium (3, 5, and 29), the observed results could be produced

through an increased activity of a normal supply of auxin. If auxin acts at the cell surface as Lundegardh (15) suggests, the full effect of higher concentration of hydrogen ions in the medium might be exerted even if the internal pH remained virtually unchanged.

Variation in the external acidity of the growth medium from pH 3.5 to pH 5.0 had little effect on the pH of the expressed sap. If the pH of expressed sap is a close approximation to the pH of the vacuole, as claimed by Chibnall and Grover (8), the results obtained in these experiments give an indication of the effect of external acidity on the pH of the vacuolar sap. Microinjection studies with amoebae have shown that the cytoplasm was strongly buffered (20) and that, although acids or alkalies in the surrounding medium readily changed the pH of the vacuoles, they did not change the pH of the cytoplasm of living cells (7). It is to be expected, therefore, that external acidity would have even less effect on the pH of the cytoplasm than on the pH of the vacuole. In the present experiment, even the pH of the vacuole varied little as compared with the pH of the medium. Apparently $[H^+]$ of strong acids do not obey the diffusion law of tending to equal concentration at equilibrium. Some kind of equilibrium between the concentration inside and the concentration outside seems to exist, but the transfer must depend on the unknown mechanism of ion transfer—the so-called active permeability. The lack of large differences in the pH of expressed sap from plants grown in different hydrogen-ion concentrations supports the view that the penetration of strong acids into cells depends on an active type of permeability.

Direct evidence that the penetration of acids into plant cells is decreased by the growing of plants in an acid medium is provided by the fact that roots from the most acid solutions were able to tolerate a higher than average concentration of hydrochloric and acetic acids for a given period of time. Under the section on results, it was suggested that this decrease in penetration of acid might be attributed to a decreased permeability of the cells grown in highly acid media. The type of permeability, if it can be called such, that is decreased is probably of the active sort referred to in the preceding paragraph. It may be possible, of course, that high concentrations of hydrochloric acid create a passive permeability to itself, in which case some change in the plasma membrane would be necessary to protect the membrane from the action of the acid.

The absence of any increase in the permeability to thiourea of the root cells from the various hydrogen-ion concentrations is an indication that passive permeability to polar substances and to small molecules in general is not decreased by growth in acid media, as, for the most part, a change in permeability to one such substance is attended by a similar change toward others. This result supports the view that if, as has been suggested, the penetration of strong acid into meristem cells is reduced by adaptation to an acid medium, it is the active type of permeability which is affected. Such a reduction does not preclude, however, a further adaptation of an increased resistance on the part of the plasma membrane, which the extraordinary tolerance of hydrochloric acid by meristem cells would seem to demand.

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THE OCCURRENCE OF *FUSARIUM* SPECIES IN CANADA

II. PREVALENCE AND TAXONOMY OF *FUSARIUM* SPECIES IN CEREAL SEED[1]

By W. L. Gordon[2]

Abstract

During the present investigation, a total of 1579 seed samples of wheat, 1042 of barley, and 1152 of oats (100 kernels per sample), were examined microbiologically for the presence of *Fusarium*. Of these samples, 402 of wheat, 513 of barley, and 636 of oats originated in the seed inspection districts of Eastern Canada, and 1177 of wheat, 529 of barley, and 516 of oats in those of Western Canada. Isolates of *Fusarium* were obtained from approximately 41.8% of the samples of wheat (1.5% of the kernels), 76.2% of the samples of barley (3.9% of the kernels), and 79.6% of the samples of oats (5.7% of the kernels) that originated in Eastern Canada, whereas only 13.8% of the samples of wheat (0.2% of the kernels), 36.3% of the samples of barley (0.7% of the kernels), and 38.9% of the samples of oats (1.1% of the kernels) from Western Canada yielded *Fusarium*. In classifying the different wild types of *Fusarium* that were obtained from cereal seed the system of taxonomy and nomenclature of Wollenweber and Reinking was chiefly followed, but certain sections of the genus were revised extensively, partly in accordance with Snyder and Hansens' concept of species in this genus. Four new combinations are proposed, namely *F. compactum* (Wr.) n. comb., *F. lateritium* Nees emend. Snyder & Hansen forma *cajani* (Padwick) n. comb., *F. lateritium* Nees emend. Snyder & Hansen forma *crotalariae* (Padwick) n. comb., and *F. oxysporum* Schlecht. emend. Snyder & Hansen var. *redolens* (Wr.) n. comb. A total of 16 species and varieties of *Fusarium*, classified in nine sections of the genus, was isolated from cereal seed during this investigation. These species and varieties are, namely, *F. poae* (Pk.) Wr., *F. sporotrichioides* Sherb., *F. avenaceum* (Fr.) Sacc., *F. arthrosporioides* Sherb., *F. semitectum* Berk. & Rav., *F. equiseti* (Cda.) Sacc., *F. acuminatum* Ell. & Ev., *F. culmorum* (W. G. Sm.) Sacc., *F. graminearum* Schwabe, *F. sambucinum* Fuckel, *F. sambucinum* var. *coeruleum* Wr., *F. lateritium* Nees emend. Snyder & Hansen, *F. moniliforme* Sheld. emend. Snyder & Hansen, *F. oxysporum* var. *redolens* (Wr.) n. comb., and *F. solani* (App. & Wr.) Wr. emend. Snyder & Hansen. *F. poae*, *F. avenaceum*, and *F. acuminatum* were most frequently isolated. *F. concolor* Rg. and *F. sambucinum* f. 6 Wr., that were previously recorded from cereal seed in Manitoba, and three additional species, namely, *F. dimerum* Penz., *F. merismoides* Cda., and *F. nivale* (Fr.) Ces., that may be encountered in the future among isolates from cereal seed in Canada, were also included in this study.

Introduction

In Part I of this series of papers (13) data were presented on the incidence of *Fusarium* species in farm samples of cereal seed produced in Manitoba during the crop years 1937 to 1942. These data showed that *Fusarium* species were encountered in a relatively large percentage of the total number of samples of cereal seed examined but in a relatively small percentage of the kernels. The majority of the species that were isolated from the seed proved to be the same as those species which had been previously shown to be associated with root rots of cereals in Manitoba (12).

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Although the production in Canada of the cereal crops, wheat, oats, and barley, may be considered to be centered in Western Canada, particularly in the prairie provinces, they are also produced to a lesser extent in Eastern Canada. Seed production of these crops therefore takes place under diverse environmental conditions. Whether or not cereal seed produced under the environmental conditions prevailing in Eastern Canada would yield on microbiological examination the same species of *Fusarium* as cereal seed produced in Western Canada seemed worthy of investigation.

This investigation was carried on at Winnipeg, Man. as part of a co-operative project on the microflora of cereal seed in relation to common root rot of cereal crops in Canada. The results of this investigation, dealing particularly with the identity and classification of the isolates of *Fusarium* obtained from the samples of cereal seed, and their prevalence in seed samples from Eastern Canada as compared with those from Western Canada, are presented in this paper.

Materials and Methods

Number and Source of Seed Samples

The samples of cereal seed examined microbiologically during the course of this investigation were obtained from the crops harvested in Canada during each of the five years 1939 to 1943. The majority of these seed samples were secured through the courtesy of the Plant Products Division, Production Service, Canada Department of Agriculture. The number of seed samples from this source amounted to 1424 of wheat, 944 of barley, and 984 of oats. A smaller number of seed samples from Elite and Registered seed stocks were provided by the Canadian Seed Growers' Association, Ottawa, during each of the three years 1941 to 1943. From this seed source were obtained 155 samples of wheat, 98 of barley, and 168 of oats. Some of the samples from both seed sources originated in each of the eight seed inspection districts of Canada, four of which, namely, Maritime Provinces, Quebec, Eastern Ontario, and Western Ontario are in Eastern Canada, and the remaining four in Western Canada, corresponding essentially to the provinces of Manitoba, Saskatchewan, Alberta, and British Columbia. Of the various seed samples examined, 402 of wheat, 513 of barley, and 636 of oats were obtained from the seed inspection districts of Eastern Canada, and 1177 of wheat, 529 of barley, and 516 of oats originated in the seed inspection districts of Western Canada. A total, therefore, of 1579 seed samples of wheat, 1042 of barley, and 1152 of oats were available for microbiological examination. Although these samples, obtained from Certified, Registered, and Elite classes of cereal seed, were undoubtedly superior in genetic purity to the average seed produced commercially in Canada, they are considered to be quite representative of commercial cereal seed with respect to infestation by *Fusarium*.

Method of Isolation

Details of the method employed in the surface sterilization and plating of the seed have been published by Greaney and Machacek (17) to whom the

PLATE I

Fig. 1. The type of trays on which cultures of *Fusarium* were kept so that their growth characteristics could be observed readily.

writer is indebted for the collection and plating of the seed samples. Briefly, however, the method employed was as follows. One hundred kernels were taken at random from each of the seed samples of wheat, barley, and oats, surface sterilized in a solution of ethyl alcohol and mercuric chloride, washed in sterile water, and plated on potato dextrose agar in Petri dishes. After incubation of the Petri dish cultures for eight days at a temperature of from 24° to 28°C., the colonies of *Fusarium* that developed from the kernels were transferred to potato dextrose agar slants for further study.

Culture Technique

After a preliminary microscopic examination of the isolates of *Fusarium* that were obtained from the seed each year during this investigation, several cultures of each apparently different type of isolate were retained for further examination and identification. These different types of isolates, which were obtained directly from seed that was produced in nature, may be referred to as 'wild types'. They were maintained in culture by consecutive monoconidial or hyphal tip transfers. Identification of the different wild-type isolates was based upon the morphological and cultural characters exhibited by them when grown on potato sucrose agar slants (2% sucrose, 2% agar). These cultures were maintained in the laboratory, on the type of trays shown in Fig. 1, so that each culture could receive approximately the same amount of light, and could be examined readily. This laboratory was illuminated at an intensity of 50 ft-c. by fluorescent lighting and a small amount of daylight for approximately 10 hr. each day. The temperature of the laboratory varied from 20° to 24°C.

Determination of Growth Rates

Growth rates of the wild types of each of the species, varieties, or forms of *Fusarium* encountered among the isolates were determined on potato sucrose agar (pH 6.5) in Petri dishes that were kept in an oven maintained at a temperature of 25° to 26°C. The rate of growth of each species, variety, or form of *Fusarium* is expressed as the average diameter in centimeters attained by eight monoconidial colonies at the end of four days.

Measurements and Drawings

For measuring and drawing purposes, conidia were taken from wild-type cultures of each species, variety, and form of *Fusarium* when approximately two weeks old. In order to be certain that these conidia were actually formed by the wild-type cultures, and not by mutants that may have originated in them, several monoconidial cultures, for comparison with wild-type cultures were made from each lot of conidia. The limits of the range in length and width of the conidia are given in microns. Drawings of conidia and chlamydospores were made with the aid of a camera lucida, at a microscope magnification of 1000×.

Photographs in Color

Ansco color film, tungsten type, 4 × 5 in. in size, was employed in photographing the monoconidial cultures of the wild type of each species, variety,

or form of *Fusarium*. Potato sucrose agar, slanted in 1 × 8 in. test tubes, was used as a medium for all cultures photographed. The age of the cultures of the various species, varieties, and forms of *Fusarium* when photographed varied from 8 to 12 days, depending on their respective growth rates.

Percentages of the Seed Samples and Kernels of Wheat, Barley, and Oats that Yielded *Fusarium* Isolates

Fusarium isolates were obtained from relatively large percentages of the total number of seed samples of wheat, of barley, and of oats. Approximately 21% of the 1579 seed samples of wheat, 56% of the 1042 samples of barley, and 61% of the 1152 samples of oats yielded isolates. On the other hand, relatively small percentages of the total number of kernels of wheat, of barley, and of oats were found to be infested. Only 0.54% of the 157,900 wheat kernels, 2.27% of the 104,200 barley kernels, and 3.63% of the 115,200 oat kernels yielded *Fusarium* isolates.

Fusarium was found to be more commonly seed-borne in cereal crops in Eastern Canada than in Western Canada, as indicated by the data presented in Table I. In this table are shown the percentages of the total number of samples and of kernels of wheat, of barley, and of oats from Eastern Canada and from Western Canada that yielded *Fusarium* isolates. It is also evident from the data given in Table I that *Fusarium* was harbored more frequently by the seed of oats and barley than by the seed of wheat in both geographic areas mentioned.

TABLE I

THE PERCENTAGES OF THE TOTAL NUMBER OF SAMPLES AND KERNELS OF WHEAT, OF BARLEY, AND OF OATS FROM EASTERN CANADA AND FROM WESTERN CANADA THAT YIELDED *Fusarium* ISOLATES

Origin of samples	Percentages of samples and of kernels yielding *Fusarium* isolates					
	Wheat		Barley		Oats	
	Samples	Kernels	Samples	Kernels	Samples	Kernels
Eastern Canada	41.79	1.45	76.22	3.88	79.56	5.67
Western Canada	13.76	0.23	36.29	0.70	38.95	1.11

Present Status of *Fusarium* Classification

At the present time two complete systems of nomenclature and taxonomy are available for naming and classifying *Fusarium* isolates. These are, namely, the detailed system of Wollenweber and Reinking (50) and the simplified system of Snyder and Hansen (37, 38, 39).

Following the publication of the "Grundlagen einer Monographie der Gattung *Fusarium* (Link)" by Appel and Wollenweber (1) in 1910, the classification of the species, varieties, and forms of *Fusarium* gradually evolved into a widely recognized system of taxonomy. This system was presented by Wollenweber and Reinking (50) in "Die Fusarien", and is often referred to

as the "Wollenweber system". According to this system of taxonomy, the genus *Fusarium* is divided into 16 named sections in which a total of 65 species, 55 varieties, and 22 forms of *Fusarium* are differentiated. While this division of the genus into sections, species, and the like was a marked advance in *Fusarium* classification, it failed to meet with unqualified approval owing to the difficulties that were still encountered in attempting to classify specific isolates of *Fusarium* (37).

A much simplified system of *Fusarium* nomenclature and taxonomy was proposed by Snyder and Hansen (37, 38, 39) during the period 1940 to 1945, as a result of their extensive investigations of the variability exhibited in culture by species, varieties, and forms of *Fusarium*. The section Elegans of the genus was the first to be revised according to their concept of species (37). Simplification of this section was achieved by emending the description of a single species, namely, *F. oxysporum* Schlecht., to agree with the description of the section given by Wollenweber (43). The 10 species, 18 varieties, and 12 forms in the section Elegans were placed in this one species, on the sole basis of morphology. Twenty-five pathogens of the section were then classified as "formae" of *F. oxysporum* on the basis of pathogenicity. In 1941 Snyder and Hansen (38) made a similar revision of the section Martiella, with a consequent reduction of the three species, seven varieties, and three forms of *Fusarium* in this section to a single species, namely, *F. solani* (Mart.) App. & Wr. emend. Snyder & Hansen. Five pathogens of this section were classified as "formae" of *F. solani*. In a later publication, Snyder and Hansen (39) considered *F. argillaceum* (Fr.) Sacc., the only species in the section Ventricosum, to be a synonym of *F. solani*. The descriptions of section Ventricosum and of *F. solani* were therefore merged.

The third, and final, part of the comprehensive revision of the genus by Snyder and Hansen (39) appeared in 1945 and covered the remaining 13 sections. As a result of their complete revision of the genus the 16 sections, 65 species, 55 varieties, and 22 forms of *Fusarium*, previously differentiated by Wollenweber and Reinking (50), were reduced to eight species, no varieties, and 34 forms (mostly of *F. oxysporum*). Two doubtful species were also listed.

An examination of the more recent literature on *Fusarium* will reveal that each system of classification has its adherents. It will be evident as well that the simplified classification of the section Elegans devised by Snyder and Hansen has gained more general approval than their similar revision of the other sections of the genus.

A comprehensive review of the literature on the taxonomic problem in *Fusarium* has recently been presented by Miller (25, 26). From the results of his own investigations on the variability of *Fusarium* species in culture, he concluded that the descriptions of many *Fusarium* species are probably based to a large extent on cultural variants of the types found in nature. He was of the opinion, as well, that many of the uncertainties that arise in the identification of *Fusarium* isolates could be avoided by basing species descriptions on the characters of the wild types.

Classification of the Isolates from Cereal Seed

Neither the system of *Fusarium* taxonomy and nomenclature of Wollenweber and Reinking (50) nor the system of Snyder and Hansen (37, 38, 39) was found to be satisfactory, *in toto*, for the classification of the different wild-type isolates of *Fusarium* that were obtained from cereal seed. Certain of the isolates were not identifiable with certainty according to the system of Wollenweber and Reinking (50), as they appeared to fit the description of two or more species equally well. On the other hand, certain other isolates that were readily distinguishable as to species, variety, or form by the system of Wollenweber and Reinking would have been grouped together in the same species according to the system of Snyder and Hansen.

The system of taxonomy followed more closely, however, for the classification of the wild-type isolates of *Fusarium* is basically that of Wollenweber and Reinking (50), as presented by them in "Die Fusarien". The sectional divisions of the genus, characteristic of their system of classification, have been retained with one exception, namely, the section Ventricosum, which is considered to be a synonym of the section Martiella. This modification in the system of Wollenweber and Reinking is in line with the revision of the section Ventricosum proposed by Snyder and Hansen (39). Certain sections of the genus, particularly Lateritium, Liseola, Elegans, and Martiella, were also modified by the adoption of the revisions of these sections as a whole or in part by Snyder and Hansen (37, 38, 39). Less inclusive changes in the system of taxonomy of Wollenweber and Reinking (50) than those proposed by Snyder and Hansen were effected in other sections of the genus when they appeared to be required in order to make a more practical classification. It should be pointed out, however, that these changes were not instituted solely as a result of a study of the isolates of *Fusarium* that were obtained from cereal seed in Canada. Several thousand isolates of *Fusarium* additional to those from cereal seed were examined. These isolates were obtained from various host plants and from soil in Canada. Furthermore, occasional cultures of *Fusarium* that had been isolated in other countries from a wide variety of hosts were available during the course of this investigation for comparison with isolates from Canada. These cultures of *Fusarium* were received from Argentina, Australia, Belgian Congo, Gold Coast, Great Britain, Greece, Holland, India, Kenya-Uganda-Tanganyika territory, Malay States, Mauritius, New Zealand, Nigeria, Nyasaland, Palestine, St. Helena, Southern Rhodesia, Sudan, United States, and Uruguay.

According to the method of classification adopted by the writer, a total of 16 species and varieties of *Fusarium* was identified among the isolates obtained from the 1579 samples of wheat (157,900 kernels), 1042 samples of barley (104,200 kernels), and the 1152 samples of oats (115,200 kernels) during this investigation. These species and varieties represented nine sections of the genus, namely, Sporotrichiella, Roseum, Arthrosporiella, Gibbosum, Discolor, Lateritium, Liseola, Elegans, and Martiella.

An additional four species, and one form of *Fusarium* that were not isolated from cereal seed during this investigation, were included in the taxonomic and cultural studies. *F. concolor* Rg. (section Arthrosporiella) and *F. sambucinum* Fuckel form 6 Wr. (section Discolor) were previously encountered among isolates of *Fusarium* obtained from farm samples of cereal seed in Manitoba (13). *F. nivale* (Fr.) Ces. (section Arachnites), although not isolated as yet from cereal seed in Canada, is recognized to be a seed-borne pathogen of cereals, particularly in Europe (50). *F. dimerum* Penz. and *F. merismoides* Cda. (section Eupionnotes) have been isolated from cereal soils in Canada and they may possibly be encountered in the future among isolates from cereal roots or seed.

Keys to the sections, species, varieties, and forms of the genus *Fusarium*, according to the system of nomenclature and taxonomy of Wollenweber and Reinking (50), may be found in "Die Fusarien". In addition, a key to the important species of *Fusarium* that occur on the Gramineae in the United States has recently been published by Sprague (40). It is based on cultural as well as morphological characteristics, and includes, incidentally, all the more important species that have been found in Canada to be associated with the seed of cereals.

The synonymy of the species, varieties, and forms of *Fusarium* included in this paper is largely confined to those names encountered in the more recent literature of *Fusarium*. A more complete synonymy may be found in "Fusarium-Monographie" by Wollenweber (47, 48) and in "Die Fusarien" by Wollenweber and Reinking (50).

Section Eupionnotes Wr.

Wollenweber, Phytopath. 3: 206 and 219. 1913.
Wollenweber, Fusarium-Monographie, pp. 291-292. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 18-20. 1935.

Two species of *Fusarium* classified in this section have been isolated, as previously mentioned, from cereal soils but not from cereal seed in Canada. These two species are *F. dimerum* Penz. and *F. merismoides* Cda.

Wollenweber (47), in 1931, reduced the rank of *F. pusillum* Wr. to that of a variety of *F. dimerum*, namely, *F. dimerum* var. *pusillum* Wr. This variety, according to Wollenweber (47) differed from *F. dimerum* in having conidia that were often nonseptate. As septation of conidia in *F. dimerum* has been observed to be a highly variable character it is improbable that the variety *pusillum* can be distinguished from it with certainty. The name *F. dimerum* var. *pusillum* is therefore considered to be synonymous with *F. dimerum*.

F. merismoides var. *chlamydosporale* Wr. and *F. merismoides* var. *crassum* Wr., were distinguished from *F. merismoides* by Wollenweber (47) in 1931. The variety *chlamydosporale* was stated to differ from *F. merismoides* in having larger conidia and a more profuse production of chlamydospores; the variety *crassum* in having wider conidia and larger chlamydospores. Variation in size of conidia and in size and number of chlamydospores has been found to be

sufficiently great in *F. merismoides* as to render these varieties indistinguishable from it. The variety names *chlamydosporale* and *crassum* of *F. merismoides* are therefore relegated to the synonymy of this species.

FUSARIUM DIMERUM Penz. (Fig. 24)
Penzig, Fungi agrum., Michelia, 2: 484-485. 1882.
Wollenweber and Reinking, Die Fusarien, p. 26. 1935.

Syn. *F. dimerum* Penz. var. *pusillum* Wr.
Wollenweber, Fusarium-Monographie, p. 305. 1931.
Wollenweber and Reinking, Die Fusarien, p. 26. 1935.

F. episphaeria (Tode) Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 662. 1945.

Conidia and Chlamydospores (Fig. 3)

Conidia, 1(0–3)–septate; 0–sept. 6.4—10.6 × 2.3–3.4
1–sept. 10.6–17.0 × 2.5–3.6
2–sept. 10.6–19.8 × 2.5–2.9
3–sept. 17.0–21.2 × 2.7–3.2

Chlamydospores intercalary, singly, in pairs, or in chains. Growth rate 2.7 cm. Perfect stage unknown.

Habitats in Canada

F. dimerum has been occasionally isolated from cereal soils in Manitoba. It was previously recorded by Bisby *et al.* (4) from soil and butter in Manitoba.

FUSARIUM MERISMOIDES Cda. (Fig. 23)
Corda, Icones fungorum. II: 4. 1838.
Wollenweber and Reinking, Die Fusarien, pp. 24-25. 1935.

Syn. *F. merismoides* var. *chlamydosporale* Wr.
Wollenweber, Fusarium-Monographie, p. 308. 1931.
Wollenweber and Reinking, Die Fusarien, p. 25. 1935.

F. merismoides Cda. var. *crassum* Wr.
Wollenweber, Fusarium-Monographie, p. 308. 1931.
Wollenweber and Reinking, Die Fusarien, p. 25. 1935.

F. episphaeria (Tode) Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 662. 1945.

Conidia and Chlamydospores (Fig. 2)

Conidia, 3(1–5)–septate; 2–sept. 25.4–36.5 × 3.8–4.2
3–sept. 27.6–40.3 × 3.2–4.7
5–sept. 36.1–40.3 × 4.2–4.5

Chlamydospores intercalary, singly, in pairs, or in chains. Growth rate 0.84 cm., which is the slowest growth rate of all the species, varieties, and forms of *Fusarium* studied. Perfect stage unknown.

Although *F. merismoides* is not known to be pathogenic to cereals, Wollenweber (47) records a variety of this species from the seed of wheat and rye

and Bennett (2) found it repeatedly on the basal parts of wheat and oats and on the seed of barley.

Habitats in Canada

F. merismoides has been isolated from cereal soils in Manitoba. Bisby *et al.* (4) previously reported that a few isolates of this species were obtained from soil in Manitoba.

Section Arachnites Wr.

Wollenweber, Ann. Mycol. 15: 2. 1917.
Wollenweber, Fusarium-Monographie, p. 314. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 42-43. 1935.

Owing to the variability in size of conidia observed to occur in cultures of *F. nivale* (Fr.) Ces., *F. nivale* var. *majus* Wr. and its perfect stage *Calonectria graminicola* (Berk. & Brme.) Wr. var. *neglecta* Krampe are considered to be synonymous with *F. nivale* (Fr.) Ces. and with *C. nivalis* Schaffnit, respectively. *F. nivale* var. *majus* was named and illustrated by Wollenweber (46) in 1930 and was reported by him (47) in 1931 to differ from *F. nivale* in having larger conidia. A description of the var. *majus* was provided by Wollenweber and Reinking (50) in 1935.

FUSARIUM NIVALE (Fr.) Ces. (Fig. 25)
Wollenweber, Fusarium-Monographie, pp. 317-318. 1931.
Wollenweber and Reinking, Die Fusarien, p. 44. 1935.

Syn. *F. nivale* (Fr.) Ces. var. *majus* Wr.
Wollenweber, Fusaria Autographice Del., No. 882. 1930.
Wollenweber, Fusarium-Monographie, p. 319. 1931.
Wollenweber and Reinking, Die Fusarien, p. 45. 1935.

F. nivale (Fr.) Ces. emend. Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 662. 1945.

F. nivale (Fr.) Ces. emend. Snyder & Hansen f. *graminicola* (Berk. & Brme.) Snyder and Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 662. 1945.

Cultures of the wild type of *F. nivale* were available from two sources for comparison with the isolates of *Fusarium* that were obtained from cereal seed during this investigation. This species was isolated from the seed of barley that was produced in Scotland and from head blight of wheat collected in Holland. Cultures of this species of Canadian origin have not been seen by the writer.

Conidia (Fig. 4)

Conidia, 3(0–6)–septate; 0–sept. 8.5–10.6 × 2.6–3.2
1–sept. 10.6–19.1 × 3.2–4.5
2–sept. 10.1–21.2 × 3.2–4.5
3–sept. 17.0–25.4 × 3.2–5.9
4–6–sept. 19.8–30.7 × 3.4–6.2

Chlamydospores absent. Growth rate 1.3 cm.

Habitats in Canada

This species was reported by Dahl (9) in 1934 to be associated with snow mold of turf grasses in the United States and Canada. It was also recorded by Sprague (40) in 1950 on *Agrostis palustris* Huds. (Washington bent grass) from British Columbia and Ontario and on *Agrostis tenuis* Sibth. (Colonial bent grass) from Ontario.

CALONECTRIA NIVALIS Schaffnit
Schaffnit, Mykol. Centralbl. 2: 257. 1913.
Petch, Trans. Brit. Mycol. Soc. 27: 150-151. 1945.

Syn. *C. graminicola* (Berk. & Brme.) Wr.
Wollenweber, Phytopath. 3: 34. 1913.
Wollenweber, Fusarium-Monographie, pp. 318-319. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 44-45. 1935.

C. graminicola (Berk. & Brme.) Wr. var. *neglecta* Krampe
Krampe, Angew. Bot. 8: 252. 1926.
Wollenweber, Fusarium-Monographie, p. 319. 1931.
Wollenweber and Reinking, Die Fusarien, p. 45. 1935.

C. graminicola Wr.
Plant Pathology Committee, List of Common British Plant Diseases, p. 7. 1944.

C. nivale (Fr.) Snyder & Hansen
Snyder and Hansen, Am. J. Botany, 32: 663. 1945.

C. nivale (Fr.) Snyder & Hansen f. *graminicola* (Berk. & Brme.) Snyder & Hansen

Snyder and Hansen, Am. J. Botany, 32: 663. 1945.

Petch (31), in 1945, advanced the view that the binomial *C. nivalis* Schaffnit should be employed to denote the perfect stage of *F. nivale* rather than *C. graminicola* (Berk. & Brme.) Wr. or *C. graminicola* Wr. His recommendation was accepted by the Subcommittee of the Plant Pathology Committee of the British Mycological Society (41).

Perithecia of *C. nivalis* Schaffnit have not been recorded from nature in Canada. They have been induced to form, however, in single ascospore and in single conidial cultures. This fungus was reported by the writer in 1948 (42) to be bisexual and self-fertile.

Section Sporotrichiella Wr.

Wollenweber, apud Lewis in Maine Agr. Expt. Sta. Bull. 219:256. 1913.
Wollenweber and Reinking, Die Fusarien, pp. 45-47. 1935.
Wollenweber, Fusarium-Monographie II, pp. 125-127. 1944-45.

Two species of *Fusarium* that belong in this section of the genus, namely, *F. poae* (Pk.) Wr. and *F. sporotrichioides* Sherb., were represented among the isolates obtained from cereal seed.

F. citriforme Jamal. was described as a new species of the section Sporotrichiella by Jamalainen (19) in 1943. This species was differentiated from *F. poae* chiefly on the basis of the shape and size of the microconidia as in other respects it resembled *F. poae* closely. Owing to the variation in the size and shape observed to occur in the microconidia and macroconidia of wild type isolates of *F. poae*, it is considered to be doubtful whether *F. citriforme* can be distinguished from *F. poae* with certainty. For this reason, it seems best to consider the name *F. citriforme* to be a synonym of *F. poae*.

FUSARIUM POAE (Pk.) Wr. (Fig. 26)
Wollenweber and Reinking, Die Fusarien, p. 47. 1935.
Wollenweber, Fusarium-Monographie II, pp. 127-128. 1944-45.

Syn. *F. citriforme* Jamal.
Jamalainen, Staat, Landw. Versuchstat. Veröffentl. 123: 8-11. 1943.
Wollenweber, Fusarium-Monographie II, p. 128. 1944-45.

F. tricinctum (Cda.) Sacc. emend. Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 663. 1945.

F. tricinctum (Cda.) Sacc. emend. Snyder & Hansen f. *poae* (Pk.) Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 663. 1945.

Conidia and Chlamydospores (Fig. 5)

Microconidia, 0–sept. (most common) 5.3–10.6 × 3.7–7.8
1–sept. 10.6–14.8 × 6.4–7.4
2–sept. (occasional) 15.3 × 6.6
Macroconidia, 3–sept. 19.1–38.2 × 3.8–5.7
4–sept. (occasional) 31.8–38.2 × 4.2–5.3

Chlamydospores intercalary, in singles, pairs, knots, or in chains. Growth rate 7.6 cm.; relatively fast growing in culture. Of the various species that were isolated from cereal seed, the rate of growth of *F. poae* was only exceeded by that of *F. graminearum* Schwabe and *F. culmorum* (W. G. Sm.) Sacc. (section Discolor).

Prevalence in Cereal Seed in Canada

F. poae was the most commonly isolated species of *Fusarium* from the samples of wheat, oats, and barley that originated in Eastern and in Western Canada. It was, however, encountered in a greater proportion of the samples from Eastern Canada than in those from Western Canada. In the samples that originated in Eastern Canada, it was encountered in 118 (303 kernels) of wheat, in 324 (1428 kernels) of barley, and in 484 (3351 kernels) of oats; in the samples from Western Canada, it was encountered in 40 (99 kernels) of wheat, in 111 (171 kernels) of barley, and in 169 (470 kernels) of oats.

FUSARIUM SPOROTRICHIOIDES Sherb. (Fig. 27)
Sherbakoff, N.Y. Cornell Agr. Expt. Sta. Memoir, 6: 183-186. 1915.
Wollenweber and Reinking, Die Fusarien, pp. 48-49. 1935.
Wollenweber, Fusarium-Monographie II, pp. 129-130. 1944-45.

Syn. *F. tricinctum* (Cda.) Sacc. emend. Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 663. 1945.

F. tricinctum (Cda.) Sacc. emend. Snyder & Hansen f. *poae* (Pk.) Snyder & Hansen pr. p.

Snyder and Hansen, Am. J. Botany, 32: 663. 1945.

Conidia and Chlamydospores (Fig. 6)

Microconidia, 0–sept. 6.4–9.5 × 4.2–4.9
1–sept. 10.6–17.0 × 4.2–5.9
Macroconidia, 3–sept. 27.6–40.3 × 3.2–4.5
4–sept. 29.7–36.2 × 4.0–4.5
5–sept. 36.0–46.6 × 3.8–4.2

Chlamydospores intercalary, in singles, pairs, knots, or in chains. Growth rate 3.5 cm., which is less than one half that of *F. poae*.

Prevalence in Cereal Seed in Canada

F. sporotrichioides was obtained from relatively few of the samples and kernels that were examined. In the samples obtained from Eastern Canada, it was encountered in only five (five kernels) of barley and in one (one kernel) of oats, and in the samples from Western Canada in one (one kernel) of wheat, in four (six kernels) of barley, and in one (one kernel) of oats.

Section Roseum Wr.

Wollenweber, Phytopath. 3: 32. 1913.
Wollenweber and Reinking, Die Fusarien, pp. 49-53. 1935.
Wollenweber, Fusarium-Monographie II, pp. 130-131. 1944-45.

Two species, namely, *F. avenaceum* (Fr.) Sacc. and *F. arthrosporioides* Sherb., that are classified in this section of the genus were encountered among the isolates of *Fusarium* obtained from cereal seed.

The names *F. graminum* Cda. and *F. avenaceum* var. *pallens* Wr. have been relegated to the synonymy of *F. avenaceum*. According to the description of *F. graminum* given by Wollenweber and Reinking (50), this species of *Fusarium* differs from *F. avenaceum*, in which the conidia are usually 3–5–septate, by having mostly 3 septate conidia. The description of *F. avenaceum* var. *pallens*, by the same authors (50), indicates that the conidial masses in this variety are paler in color than those of *F. avenaceum* which are usually orange colored. As these characters, namely, septation of conidia and color of conidia in mass, may vary widely in cultures of *F. avenaceum*, reliable criteria by which *F. graminum* and *F. avenaceum* var. *pallens* may be differentiated with certainty from *F. avenaceum* appear to be lacking.

FUSARIUM AVENACEUM (Fr.) Sacc. (Fig. 28)
Saccardo, Syll. Fungorum, 4: 713. 1886.
Wollenweber and Reinking, Die Fusarien, pp. 53-55. 1935.
Wollenweber, Fusarium-Monographie II, pp. 132-135. 1944-45.

Syn. *Selenosporium avenaceum* Fr.
Fries, Syst. myc. 3: 444. 1832.

F. graminum Cda.
Corda, Icones fungorum. I: 3, Fig. 59. 1837.
Wollenweber and Reinking, Die Fusarien, p. 53. 1935.
Wollenweber, Fusarium-Monographie II, pp. 131-132. 1944-45.

F. avenaceum (Fr.) Sacc. var. *pallens* Wr.
Wollenweber and Reinking, Die Fusarien, p. 56. 1935.
Wollenweber, Fusarium-Monographie II, p. 172. 1944-45.

F. roseum Lk. emend. Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 663. 1945.

F. roseum Lk. emend. Snyder & Hansen f. *cerealis* (Cke.) Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 663-664. 1945.

Conidia (Fig 7)

Conidia, 5(3–7)–septate; 3–sept. 29.7–53.0 × 3.2–4.4
4–sept. (occasional) 44.5–63.6 × 3.2–4.7
5–sept. 46.6–67.8 × 3.2–4.7
6–sept. (occasional) 63.6–65.7 × 4.2–4.4
7–sept. 67.8–72.1 × 3.6–4.2

Chlamydospores absent. Growth rate 5.4 cm. Perfect stage unknown, but according to Wollenweber (48) it is probably a *Gibberella*.

Prevalence in Cereal Seed in Canada

With the exception of *F. poae*, *F. avenaceum* was isolated from a greater proportion of the total samples, and kernels, of wheat, oats, and barley, than any other species of *Fusarium*. It was more commonly seed-borne, however, in Eastern Canada than in Western Canada.

In the samples obtained from Eastern Canada, *F. avenaceum* was encountered in 86 (226 kernels) of wheat, in 171 (445 kernels) of barley, and in 111 (167 kernels) of oats; in the samples from Western Canada this species was encountered in 19 (26 kernels) of wheat, in 47 (69 kernels) of barley, and in 20 (31 kernels) of oats.

FUSARIUM ARTHROSPORIOIDES Sherb. (Fig. 29)
Sherbakoff, N.Y. Cornell Agr. Expt. Sta. Memoir, 6: 175-179. 1915.
Wollenweber and Reinking, Die Fursarien, pp. 56-57. 1935.
Wollenweber, Fusarium-Monographie II, pp. 173-174. 1944-45.

Syn. *F. roseum* Lk. emend. Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 663. 1945.

Conidia (Fig. 8)

Conidia, 0–7–septate; 0–sept. 7.4–17.0 × 2.3–4.4
1–sept. 19.1–41.3 × 3.2–4.2
2–sept. 27.6–44.5 × 3.4–5.5
3–sept. 29.7–49.8 × 3.2–5.9
5–sept. 44.5–65.7 × 4.0–4.4
7–sept. 72.4–82.7 × 4.6–5.3

Chlamydospores absent. Growth rate 4.7 cm.

F. arthrosporioides would appear to be seldom associated with cereal seed in Canada. Only a single isolate of this species, from a sample of wheat that originated in Eastern Canada, was obtained.

Section Arthrosporiella Sherb.

Sherbakoff, N.Y. Cornell Agr. Expt. Sta. Memoir, 6: 161. 1915.
Wollenweber, Fusarium-Monographie, pp. 322-324. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 57-58. 1935.

Two species, namely, *F. concolor* Rg. and *F. semitectum* Berk. & Rav., that are classified in this section of the genus, have been isolated from cereal seed in Canada. *F. concolor* was not encountered among the isolates of *Fusarium* from cereal seed during this investigation but it has been previously reported by Gordon (13) from barley seed in Manitoba.

The names *F. semitectum* var. *majus* Wr. and *F. diversisporum* Sherb. are considered to be synonyms of *F. semitectum*. According to the descriptions of *F. semitectum* var. *majus* and *F. diversisporum* given by Wollenweber and Reinking (50), the variety *majus* differs from *F. semitectum* in having conidia with higher septation and *F. diversisporum* differs by the occasional formation of conidia in sporodochia. Both of these characters, namely, number of septa in the conidia and formation of conidia in sporodochia, have been observed to be sufficiently variable in *F. semitectum* that the separation of *F. semitectum* var. *majus* and *F. diversisporum* from it does not appear to be justified.

Fusarium concolor Rg. (Fig. 30)
Wollenweber and Reinking, Die Fusarien, p. 60. 1935.

Syn. *F. roseum* Lk. emend. Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 663. 1945.

Conidia and Chlamydospores (Fig. 9)

Conidia, 1–5–septate; 1–sept. 12.7–24.4 × 3.4–4.2
3–sept. 29.3–48.8 × 3.6–4.7
5–sept. 40.3–50.9 × 4.0–4.2

Chlamydospores, intercalary and terminal. Growth rate 4.1 cm.

Fusarium semitectum Berk. & Rav. (Fig. 31)
Berkeley, Notices of North American fungi. Grevillea, 3: 98. 1875.
Wollenweber, Fusarium-Monographie, pp. 324-325. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 58-59. 1935.

Syn. *F. semitectum* Berk. & Rav. var. *majus* Wr.
Wollenweber, Fusarium-Monographie, pp. 325-327. 1931.
Wollenweber and Reinking, Die Fusarien, p. 59. 1935.

F. diversisporum Sherb.
Sherbakoff, N.Y. Cornell Agr. Expt. Sta. Memoir, 6: 161-166. 1915.
Wollenweber, Fusarium-Monographie, pp. 327-328. 1931.
Wollenweber and Reinking, Die Fusarien, p. 61. 1935.

F. roseum Lk. emend. Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 663. 1945.

Conidia and Chlamydospores (Fig. 10)

Conidia, 0–7–septate; 0–sept. 7.4–9.5 × 3.6–4.7
1–sept. 14.8–17.0 × 3.6–6.4
2–sept. (occasional) 14.8 × 3.6
3–sept. 18.2–29.7 × 3.2–5.3
4–sept. 31.8–36.0 × 3.2–5.3
5–sept. 31.8–44.5 × 4.1–4.9
7–sept. 38.2–46.3 × 5.5–5.7

Chlamydospores intercalary. Growth rate 6.1 cm.

Prevalence in Cereal Seed in Canada

Only the occasional isolate of *F. semitectum* has been obtained from cereal seed in Canada. A single isolate of this species was obtained from a sample of barley that originated in Eastern Canada. Previous isolations of this species were reported by Gordon (13) from occasional farm samples of common wheat, durum wheat, barley, and oats in Manitoba.

Section Gibbosum Wr.

Wollenweber, Phytopath. 3: 31. 1913.
Wollenweber, Fusarium-Monographie, pp. 328-330. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 61-62. 1935.

A number of changes have been made in the nomenclature and taxonomy of certain species and varieties of *Fusarium* in this section of the genus. These changes appear to be necessary owing to the difficulties almost constantly encountered in attempts to classify with certainty those isolates of *Fusarium* that belong in this section and which in culture at first have a peach to buff colored aerial mycelium and stroma that later becomes brown. Wollenweber and Reinking (50) classified isolates of this cultural type into one or other of two species, namely, *F. equiseti* (Cda.) Sacc. or *F. scirpi* Lamb. & Fautr., depending upon whether the curvature of the conidia was parabolic or hyperbolic, respectively. The varieties of these two species, namely, *F. equiseti* var. *bullatum* (Sherb.) Wr., *F. scirpi* var. *caudatum* Wr., and *F. scirpi* var. *filiferum* (Preuss) Wr., were delimited mainly on the basis of other conidial characters, such as septation and length of the apical cell. As curvature, septation, and length of apical cell of the conidia in the type of isolates mentioned have been found to be characters that vary greatly, there appears

to be no real difference between *F. equiseti*, *F. equiseti* var. *bullatum*, *F. scirpi*, *F. scirpi* var. *caudatum*, and *F. scirpi* var. *filiferum*. These names are therefore considered to be synonymous, but *F. equiseti* has priority as the epithet *equiseti* in this binomial was published first (7).

Wollenweber (47), in 1931, published the description of a new variety of *F. scirpi*, namely, *F. scirpi* var. *compactum* Wr., and in addition the description of a new form of this variety, namely, *F. scirpi* var. *compactum* form 1 Wr. This new form of *F. scirpi* var. *compactum* was stated to differ from the variety *compactum* by the lack of carmine color in the stroma (47). In 1935, however, Wollenweber and Reinking (50) recorded *F. scirpi* var. *compactum* f. 1 as a synonym of *F. scirpi* var. *compactum*. As *F. scirpi* var. *compactum* f. 1 does not appear to be distinguishable with certainty from *F. equiseti* either on a morphological or cultural basis, the name *F. scirpi* var. *compactum* f. 1 is considered to be a synonym of *F. equiseti*. On the other hand, *F. scirpi* var. *compactum* is readily distinguishable from *F. equiseti* in culture by the possession of carmine-red color in the stroma and by its characteristically wide, pointed conidia. Although *F. scirpi* has been merged with *F. equiseti*, the variety *compactum* is retained by raising it to specific rank with the name *F. compactum* (Wr.) n. comb. This comparatively rare species of *Fusarium* may also be differentiated from other species retained in this section of the genus by its cultural characteristics and the shape of its conidia.

Two additional varieties of *F. scirpi*, namely, *F. scirpi* var. *acuminatum* (Ell. & Ev.) Wr. and *F. scirpi* var. *longipes* Wr., are also distinguishable from *F. equiseti* by the carmine-red color in their stroma. Each of these varietal epithets, namely, *acuminatum* and *longipes*, which originally held specific rank in the genus, are now returned to this rank as *F. acuminatum* Ell. & Ev. and *F. longipes* Wr. *F. acuminatum* was described as a new species by Ellis and Everhart (11) in 1895. The description of this species was emended by Wollenweber (44) in 1914 and its rank was reduced by him (47) in 1931, when its name was changed to *F. scirpi* var. *acuminatum*. *F. longipes* was described as a new species by Wollenweber and Reinking (49) in 1925, but it was reduced to varietal rank by the same authors (50) in 1935, in the name *F. scirpi* var. *longipes*.

As a result of these changes in nomenclature and taxonomy the section Gibbosum now consists of four species, namely, *F. equiseti*, *F. compactum*, *F. acuminatum*, and *F. longipes*, and no varieties.

Gibberella intricans Wr., which was reported to be the perfect stage of *F. equiseti* var. *bullatum* by Wollenweber (47) in 1931 now becomes the perfect stage of *F. equiseti*, and *G. acuminata* Wr. which was described as the perfect stage of *F. scirpi* var. *acuminatum* by Wollenweber and Reinking (50) in 1935, becomes the perfect stage of *F. acuminatum*. The perfect stages of *F. compactum* and *F. longipes* are unknown.

Two species in this section of the genus, namely, *F. equiseti* and *F. acuminatum*, have been isolated from cereal seed in Canada.

FUSARIUM EQUISETI (Cda.) Sacc. (Fig. 32)
Saccardo, Syll. Fungorum 4, pp. 707-708. 1886.
Wollenweber and Reinking, Die Fusarien, pp. 63-64. 1935.

Syn. *Selenosporium equiseti* Cda.
Corda, Icones fungorum. II: 7, fig. 32. 1838.

F. equiseti (Cda.) Sacc. var. *bullatum* (Sherb.) Wr.
Wollenweber, Fusarium-Monographie, p. 331. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 64-65. 1935.

F. scirpi Lamb. & Fautr.
Lambotte and Fautrey apud Roumeguère in Rev. Mycol., p. 111. 1894.
Wollenweber, Fusarium-Monographie, pp. 334-335. 1931.
Wollenweber and Reinking, Die Fusarien, p. 66. 1935.

F. scirpi Lamb. & Fautr. var. *caudatum* Wr.
Wollenweber, Fusarium-Monographie, pp. 336-337. 1931.
Wollenweber and Reinking, Die Fusarien, p. 68. 1935.

F. scirpi Lamb. & Fautr. var. *compactum* f. 1 Wr.
Wollenweber, Fusarium-Monographie, p. 333. 1931.

F. scirpi Lamb. & Fautr. var. *filiferum* (Preuss) Wr.
Wollenweber, Fusarium-Monographie, pp. 337-338. 1931.
Wollenweber and Reinking, Die Fusarien, p. 69. 1935.

F. roseum Lk. emend. Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 663. 1945.

F. roseum Lk. emend. Snyder & Hansen f. *cerealis* (Cke.) Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 663-664. 1945.

Conidia and Chlamydospores (Fig. 11)

Conidia, 5(0–9)–septate; 0–sept. 6.4–16.9 × 2.5–3.8
1–sept. 10.6–23.3 × 2.5–4.2
3–sept. 25.4–50.8 × 3.6–4.6
5–sept. 33.9–67.8 × 3.6–5.3
7–sept. 53.0–76.3 × 4.2–5.7
9–sept. 74.0–80.6 × 3.6–5.3

Chlamydospores intercalary, in singles, pairs, knots, or chains. Growth rate 5.9 cm.

Prevalence in Cereal Seed in Canada

F. equiseti was found to be seed-borne in wheat, barley, and in oats in both Eastern and Western Canada. It was more commonly seed-borne in wheat and oats in Eastern Canada, and in barley in Western Canada. However, these differences were slight as this species was isolated from less than 1% of the total number of kernels of each of the three crops in each geographic area just mentioned. In the samples that originated in Eastern Canada, *F. equiseti* was encountered in 13 (18 kernels) of wheat, in 12 (17 kernels) of

barley, and in 19 (34 kernels) of oats; in the samples from Western Canada it was encountered in 32 (36 kernels) of wheat, in 25 (41 kernels) of barley, and in 18 (22 kernels) of oats.

GIBBERELLA INTRICANS Wr.

Wollenweber, Fusarium-Monographie, p. 332. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 65-66. 1935.

Syn. *G. roseum* (Lk.) Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 664. 1945.

G. intricans, the perfect stage of *F. equiseti*, has not been found on cereals or on other hosts in Canada.

FUSARIUM ACUMINATUM Ell. & Ev. (Fig. 33)

Ellis and Everhart, Proc. Acad. Sci. Philadelphia, p. 441. 1895.
Wollenweber, J. Agr. Research, 2: 269-270. 1914.

Syn. *F. scirpi* Lamb. & Fautr. var. *acuminatum* (Ell. & Ev.) Wr.
Wollenweber, Fusarium-Monographie, pp. 335-336. 1931.
Wollenweber and Reinking, Die Fusarien, p. 67. 1935.

F. roseum Lk. emend. Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 663. 1945.

F. roseum Lk. emend. Snyder & Hansen f. *cerealis* (Cke.) Snyder & Hansen pr. p.

Snyder and Hansen, Am. J. Botany, 32: 663-664. 1945.

Conidia and Chlamydospores (Fig. 12)

Conidia, 5(0–7)–septate; 0–sept. 6.4–10.6 × 3.2–4.5
1–sept. 12.7–17.0 × 3.2–3.8
3–sept. 31.8–50.9 × 3.6–4.4
4–sept. 29.7–51.9 × 3.8–4.4
5–sept. 41.3–54.1 × 3.8–4.7
7–sept. 44.5–67.8 × 3.6–5.3

Chlamydospores intercalary, often formed in knots and chains. Growth rate 4.5 cm.

Prevalence in Cereal Seed in Canada

F. acuminatum was found to be seed-borne in wheat, barley, and oats in both Eastern and Western Canada. Although this species was isolated from less than 1% of the total number of seeds of each of the three crops in both geographic areas just mentioned, it was more commonly seed-borne, particularly in barley, in Western Canada. In the samples that originated in Eastern Canada, it was encountered in 10 (11 kernels) of wheat, in 20 (24 kernels) of barley, and in 13 (20 kernels) of oats; in the samples from Western Canada, in 32 (88 kernels) of wheat, in 46 (66 kernels) of barley, and in 13 (20 kernels) of oats.

GIBBERELLA ACUMINATA Wr.

Wollenweber and Reinking, Die Fusarien, p. 68. 1935.
Wollenweber, Fusarium-Monographie II, p. 190. 1944-45.

Syn. *G. roseum* (Lk.) Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 664. 1945.

G. acuminata, the perfect stage of *F. acuminatum*, has not been found on cereals or on other hosts in Canada.

Section Discolor Wr.

Wollenweber, Phytopath. 3: 31. 1913.
Wollenweber, Fusarium-Monographie, pp. 346-349. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 69-72. 1935.

Three species, one variety, and one form of *Fusarium*, namely, *F. culmorum* (W. G. Sm.) Sacc., *F. graminearum* Schwabe, *F. sambucinum* Fuckel, *F. sambucinum* var. *coeruleum* Wr. (=*F. sambucinum* f. 1 Wr.), and *F. sambucinum* f. 6 Wr., that belong in this section of the genus have been encountered among isolates from cereal seed in Canada.

Two minor changes in the nomenclature and taxonomy were required in this section of the genus in order to classify satisfactorily the different isolates of *Fusarium* that were obtained from cereal seed. These changes involved *F. culmorum* var. *cereale* (Cke.) Wr. and *F. sambucinum* f. 1 Wr. *F. culmorum* var. *cereale* was differentiated from *F. culmorum* by Wollenweber (47), in 1931, on the basis that it possessed longer and more slender conidia. Owing to the variation in conidial size and shape that occurs in isolates of *F. culmorum*, the continued separation from it of the var. *cereale* appears to be unjustified. The name *F. culmorum* var. *cereale* is therefore considered to be synonymous with *F. culmorum*.

In the taxonomy of *Fusarium* at the present time, the term "form" is usually employed to designate a pathogenic "strain" of a species or variety. As Wollenweber (47) did not differentiate *F. sambucinum* f. 1 from *F. sambucinum* on the basis of pathogenicity to a specific host, its earlier designation by the same author (45) as *F. sambucinum* var. *coeruleum* would appear to be the more appropriate name.

FUSARIUM CULMORUM (W. G. Sm.) Sacc. (Fig. 35)

Saccardo, Syll. Fungorum, 11: 651-652. 1895.
Wollenweber and Reinking, Die Fusarien, pp. 79-81. 1935.

Syn. *F. culmorum* (W. G. Sm.) Sacc. var. *cereale* (Cke.) Wr.
Wollenweber, Fusarium-Monographie, p. 362. 1931.
Wollenweber and Reinking, Die Fusarien, p. 81. 1935.

F. roseum Lk. emend. Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 663. 1945.

F. roseum Lk. emend. Snyder & Hansen f. *cerealis* (Cke.) Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 663-664. 1945.

Conidia and Chlamydospores (Fig. 14)

Conidia, 5(3–7)–septate; 3–sept. 26.7–36.0 × 6.1–6.6
4–sept. 29.7–42.4 × 5.7–6.8
5–sept. 31.8–50.8 × 5.3–7.4
7–sept. 40.3–50.3 × 5.9–6.4

Chlamydospores intercalary. Growth rate 8.6 cm., approximately the same as that of *F. graminearum*.

Prevalence in Cereal Seed in Canada

F. culmorum was not found to be commonly seed-borne in cereal seed in either Eastern or Western Canada. Isolates of this species were obtained from relatively few kernels, and from less than 2% of the total number of samples, of each crop from each geographic area. In the samples that originated in Eastern Canada, it was encountered in four (four kernels) of wheat, in six (11 kernels) of barley, and in three (five kernels) of oats; in the samples from Western Canada it was encountered in 11 (13 kernels) of wheat, in eight (nine kernels) of barley, and in nine (16 kernels) of oats.

FUSARIUM GRAMINEARUM Schwabe (Fig. 34)

Wollenweber, Fusarium-Monographie, pp. 362-363. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 82-83. 1935.

Syn. *F. roseum* Lk. emend. Snyder & Hansen pr. p.

Snyder and Hansen, Am. J. Botany, 32: 663. 1945.

F. roseum Lk. emend. Snyder & Hansen f. *cerealis* (Cke.) Snyder & Hansen pr. p.

Snyder and Hansen, Am. J. Botany, 32: 663-664. 1945.

Conidia (Fig. 13)

Conidia, 5(2–5)–septate; 2–sept. 23.0–32.0 × 3.2–3.6
3–sept. 30.7–46.6 × 3.6–6.1
5–sept. 40.3–55.1 × 4.9–6.4

Chlamydospores, absent or rare. Growth rate 8.9 cm. This species grew more rapidly in culture than any other species, variety, or form of *Fusarium* isolated.

Prevalence in Cereal Seed in Canada

F. graminearum was rarely isolated from cereal seed produced in Western Canada. Only three isolates of this species were obtained, each from a different sample of wheat. It was apparently somewhat more commonly seed-borne in cereals in Eastern Canada as isolates were obtained from 10 samples (16 kernels) of wheat, from 27 (46 kernels) of barley, and from four (six kernels) of oats.

GIBBERELLA ZEAE (Schw.) Petch
Petch, Ann. Mycol. 34: 256-260. 1936.

Syn. *G. saubinetii* (Mont.) Sacc. pr. p.
Saccardo, Syll. Fungorum, 2: 554-555. 1883.
Wollenweber, Fusarium-Monographie, pp. 363-365. 1931.
Wollenweber and Reinking, Die Fusarien, p. 83. 1935.

G. roseum (Lk.) Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 664. 1945.

G. roseum (Lk.) Snyder & Hansen f. *cerealis* (Cke.) Snyder & Hansen
Snyder and Hansen, Am. J. Botany, 32: 664. 1945.

Perithecia of *G. zeae*, the perfect stage of *F. graminearum*, have not been observed on cereal seed in Canada. They have been reported to occur, however, on overwintered corn stalks in Manitoba by Bisby and Bailey (3) in 1923, by Gordon *et al.* (15) in 1948, and by Gordon (14) in 1949. As ascospores were found to mature relatively late during the growing season (August), it was considered improbable that they would be of much importance in the initiation of head blight of cereals in that area (14).

FUSARIUM SAMBUCINUM Fuckel (Fig. 36)
Wollenweber, Fusarium-Monographie, pp. 352-353. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 75-76. 1935.

Syn. *F. roseum* Lk. emend. Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 663. 1945.

F. roseum Lk. emend. Snyder & Hansen f. *cerealis* (Cke.) Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 663-664. 1945.

Conidia and Chlamydospores (Fig. 15)

Conidia, 5(3–5)–septate; 3–sept. 25.4–40.3 × 4.0–5.3
4–sept. 38.2–42.4 × 4.2–4.9
5–sept. 40.3–53.0 × 4.5–5.1

Chlamydospores intercalary, often in knots and chains. Growth rate 5.2 cm.

Prevalence in Cereal Seed in Canada

F. sambucinum was seldom found to be seed-borne in cereals. In the samples that originated in Eastern Canada, this species was encountered in only two (three kernels) of wheat. In the samples from Western Canada, it was encountered in only four (four kernels) of wheat, in one (one kernel) of barley, and in three (three kernels) of oats.

GIBBERELLA PULICARIS (Fr.) Sacc.
Saccardo, Michelia, 1: 43. 1877.
Wollenweber, Fusarium-Monographie, pp. 353-356. 1931.
Wollenweber and Reinking, Die Fusarien, p. 76. 1935.

Syn. *G. roseum* (Lk.) Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 664. 1945.

G. pulicaris, reported by Wollenweber (47) to be the perfect stage of *F. sambucinum*, has not been found on cereals or other hosts in Canada.

FUSARIUM SAMBUCINUM Fuckel var. COERULEUM Wr. (Fig. 37)
Wollenweber, Ann. Mycol. 15: 55. 1917.

Syn. *F. sambucinum* Fuckel f. 1 Wr.
Wollenweber, Fusarium-Monographie, p. 356. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 76-77. 1935.

F. roseum Lk. emend. Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 663. 1945.

Conidia and Chlamydospores (Fig. 16)

Conidia, 5(0–5)–septate; 0–sept. (occasional) 8.4–9.5 × 3.8–4.2
1–sept. 12.7–13.8 × 4.2–6.2
2–sept. 14.8–19.1 × 5.3–5.9
3–sept. 16.9–29.7 × 5.1–5.9
4–sept. 25.4–29.7 × 5.5–6.1
5–sept. 25.8–36.9 × 4.7–5.9

Chlamydospores intercalary. Growth rate 4.8 cm.

Prevalence in Cereal Seed in Canada

F. sambucinum var. *coeruleum* occurred rarely in cereal seed. This species was encountered in only one sample (one kernel) of barley and in two (two kernels) of oats that originated in Eastern Canada and in one (one kernel of) barley obtained from Western Canada. It was previously reported by Gordon (13) from a farm sample of common wheat seed and from a sample of oat seed in Manitoba.

GIBBERELLA PULICARIS (Fr.) Sacc. var. MINOR Wr.

G. pulicaris var. *minor* was reported by Wollenweber (47) to be the perfect stage of *F. sambucinum* f. 1 (= *F. sambucinum* var. *coeruleum*). This variety of *G. pulicaris* has not been collected on cereals or other hosts in Canada.

FUSARIUM SAMBUCINUM Fuckel form 6 Wr. (Fig. 38)
Wollenweber, Fusarium-Monographie, p. 358. 1931.
Wollenweber and Reinking, Die Fusarien, p. 78. 1935.

Syn. *F. sulphureum* Schlecht.
Schlechtendahl, Fl. Berol., p. 139. 1824.

F. roseum Lk. emend. Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 32: 663. 1945.

Conidia and Chlamydospores (Fig. 16)

Conidia, 5(0–5)–septate; 0–sept. (occasional) 8.5–16.9 × 3.4–5.7
1–sept. (occasional) 12.7–18.5 × 3.8–6.4
3–sept. 23.3–29.7 × 4.2–4.7
5–sept. 29.7–46.6 × 4.2–4.9

Chlamydospores intercalary. Growth rate 6.4 cm.

Prevalence in Cereal Seed in Canada

F. sambucinum f. 6 was not encountered among the isolates obtained from cereal seed during this investigation. A single isolate of this form of *F. sambucinum* was previously reported by Gordon (13) from a farm sample of durum wheat in Manitoba. It is a well known pathogen of potato tubers but is probably of no importance in cereal pathology.

GIBBERELLA CYANOGENA (Desm.) Sacc.

Saccardo, Syll. Fungorum, 2: 555. 1883.
Petch, Trans, Br. Mycol. Soc. 21: 280-281. 1938.

Wollenweber (47), in 1931, reported *G. pulicaris* to be the perfect stage of *F. sambucinum*. The name *G. cyanogena* was considered by the same author to be a synonym of *G. pulicaris*. Petch (30), in 1938, however, was of the opinion that *G. cyanogena* was distinct from *G. pulicaris*.

A preliminary study by the writer of the sexual phenomena associated with the production of fertile perithecia in *G. cyanogena* showed that this fungus was bisexual (hermaphroditic) and self-sterile (42). Later it was found that by mating two mycelia of *F. sambucinum* f. 6 of opposite mating type fertile perithecia resulted, and furthermore that, when crosses were made between monosporous cultures of opposite mating type of *F. sambucinum* f. 6 and *G. cyanogena*, they yielded fertile perithecia. These perithecia appeared to be morphologically identical with the perithecia that were formed when the appropriate strains of *F. sambucinum* f. 6 were mated. *G. cyanogena* was therefore considered to be the perfect stage of *F. sambucinum* f. 6 (34). As mycelia of the two mating types of *G. cyanogena* and of *F. sambucinum* f. 6 have repeatedly failed to form perithecia when mated with mycelia of *F. sambucinum*, *G. cyanogena* would appear to be distinct from *G. pulicaris*, the perfect stage of *F. sambucinum*.

G. cyanogena has not been found in nature in Canada on cereals or other hosts.

Section Lateritium Wr.

Wollenweber, Ann. Mycol. 15: 2 and 54. 1917.
Wollenweber, Fusarium-Monographie, pp. 368-370. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 86-88. 1935.

Three species and five varieties of *Fusarium*, namely, *F. lateritium* Nees, *F. lateritium* var. *majus* Wr., *F. lateritium* var. *minus* Wr., *F. lateritium* var. *mori* Desm., *F. lateritium* var. *longum* Wr., *F. lateritium* var. *uncinatum* Wr., *F. sarcochroum* (Desm.) Sacc., and *F. stilboides* Wr., were classified in this section of the genus by Wollenweber and Reinking (50). In their revision of section Lateritium in 1945, Snyder and Hansen (39) emended the description of one species, namely, *F. lateritium*, so that it would represent all the species and varieties of *Fusarium* previously differentiated in this section by Wollenweber and Reinking (50). No "formae" of *F. lateritium*, as emended, were proposed.

The relationship of *F. udum* Butl. to *F. lateritium*, as emended by Snyder and Hansen, needs clarification. *F. udum* was described as a new species by Butler (6) in 1910 in connection with a wilt disease of pigeon pea (*Cajanus indicus* Spreng.) in India. Wollenweber and Reinking (50), in 1935, attributed this disease of *C. indicus* to a *Fusarium* probably closely related to *F. vasinfectum* Atk., and they were of the opinion that *F. udum* was possibly a "forma" of *F. vasinfectum*. In their revision of the section Elegans in 1940, Snyder and Hansen (37) classified *F. udum* as a "forma" of *F. oxysporum* Schlecht., namely, *F. oxysporum* f. *udum* (Butl.) Snyder & Hansen. Padwick (29), in 1940, was able to provide additional information on the taxonomy of *F. udum* as a result of his cultural and pathogenicity studies carried on with *F. udum*, *F. vasinfectum*, and *F. lateritium* var. *uncinatum*. He concluded from the results of his studies that *F. udum* was a separate species from *F. vasinfectum*, and that the name *F. lateritium* var. *uncinatum* was a synonym of the earlier species *F. udum*. As *F. lateritium* var. *uncinatum* is also a synonym of *F. lateritium*, as emended by Snyder and Hansen, then *F. udum* may be so regarded.

Two varieties of *F. udum* were named by Padwick (29) in 1940. He proposed the name *F. udum* var. *cajani* for the wilt organism of pigeon pea (*Cajanus cajan* Millsp. = *C. indicus* Spreng.) and *F. udum* var. *crotalariae* for the wilt organism of sunn hemp (*Crotalaria juncea* L.). As these two varieties were sharply differentiated pathogenically but were indistinguishable from each other morphologically and culturally, they may now be regarded as "formae" of *F. lateritium* Nees (= *F. udum*). The names *F. lateritium* forma *cajani* (Padwick) n. comb. and *F. lateritium* forma *crotalariae* (Padwick) n. comb. are therefore proposed for *F. udum* var. *cajani* and *F. udum* var. *crotalariae*, respectively. The synonymy of each of these "formae" is as follows:

FUSARIUM LATERITIUM Nees emend. Snyder & Hansen forma CAJANI (Padwick) n. comb.

Syn. *F. udum* Butl. var. *cajani* Padwick
Padwick, Indian J. Agr. Science, 10: 877. 1940.

F. lateritium Nees var. *uncinatum* Wr. pr. p.
Wollenweber, Fusarium-Monographie, p. 375. 1931.
Padwick, Indian J. Agr. Science, 10: 875. 1940.

F. oxysporum Schlecht. emend. Snyder & Hansen f. *udum* (Butl.) Snyder & Hansen pr. p.

Snyder and Hansen, Am. J. Botany, 27: 66. 1940.

FUSARIUM LATERITIUM Nees emend. Snyder & Hansen forma CROTALARIAE (Padwick) n. comb.

Syn. *F. udum* Butl. var. *crotalariae* Padwick
Padwick, Indian J. Agr. Science, 10: 877. 1940.

F. lateritium Nees var. *uncinatum* Wr. pr. p.
Wollenweber, Fusarium-Monographie, p. 375. 1931.
Padwick, Indian J. Agr. Science, 10: 875. 1940.

F. oxysporum Schlecht. emend. Snyder & Hansen f. *udum* (Butl.) Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 27: 66. 1940.

FUSARIUM LATERITIUM Nees emend. Snyder & Hansen (Fig. 39)
Snyder and Hansen, Am. J. Botany, 32: 664. 1945.
Wollenweber, Fusarium-Monographie, pp. 370-373. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 88-90. 1935.

Syn. *F. lateritium* Nees var. *longum* Wr.
Wollenweber, Fusarium-Monographie, p. 385. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 93-94. 1935.

F. lateritium Nees var. *majus* Wr.
Wollenweber, Fusarium-Monographie, pp. 383-384. 1931.
Wollenweber and Reinking, Die Fusarien, p. 93. 1935.

F. lateritium Nees var. *minus* Wr.
Wollenweber, Fusarium-Monographie, p. 375. 1931.
Wollenweber and Reinking, Die Fusarien, p. 92. 1935.

F. lateritium Nees var. *mori* Desm.
Wollenweber, Fusarium-Monographie, pp. 376-377. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 91-92. 1935.

F. lateritium Nees var. *uncinatum* Wr.
Wollenweber, Fusarium-Monographie, p. 375. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 92-93. 1935.

F. sarcochroum (Desm.) Sacc.
Saccardo, Michelia, 1: 534. 1879.
Wollenweber, Fusarium-Monographie, pp. 386-387. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 95-96. 1935.

F. stilboides Wr.
Wollenweber, Fusarium-Monographie, p. 385. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 94-95. 1935.

F. udum Butl.
Butler, Memoir Dept. Agr. India (Botan. Series), 2: 54. 1910.

Conidia and Chlamydospores (Fig. 18)

Conidia, 3–5(0–7)–septate; 0–sept. 8.5–10.6 × 3.8–4.0
1–sept. 14.8–24.4 × 3.4–3.8
3–sept. 23.3–43.5 × 3.4–5.3
4–sept. 34.9–42.4 × 3.8–5.3
5–sept. 36.0–43.5 × 3.4–5.7
7–sept. 38.2–46.6 × 5.3–5.9

Chlamydospores typically intercalary. Growth rate 2.8 cm.

Prevalence in Cereal Seed in Canada

Rare in cereal seed; a single isolate of *F. lateritium* was obtained from a sample of oats that originated in Eastern Canada.

Snyder and Hansen (39), in 1945, made the new combination *G. lateritium* (Nees) Snyder & Hansen to represent the perfect stage of *F. lateritium* Nees emend. Snyder & Hansen, but they considered *G. baccata* (Wallr.) Sacc. to be a synonym of it. However, as the name *G. baccata*, was published by Saccardo (32) in 1883, it has priority over *G. lateritium* (39).

GIBBERELLA BACCATA (Wallr.) Sacc.
Saccardo, Syll. Fungorum, 2: 553. 1883.
Wollenweber, Fusarium-Monographie, pp. 373-375. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 90-91. 1935.

Syn. *G. lateritium* (Nees) Snyder & Hansen
Snyder and Hansen, Am. J. Botany, 32: 664. 1945.

G. baccata (Wallr.) Sacc. var. *major* Wr.
Wollenweber, Fusarium-Monographie, p. 384. 1931.
Wollenweber and Reinking, Die Fusarien, p. 93. 1935.

G. baccata (Wallr.) Sacc. var. *moricola* (DNtrs.) Wr.
Wollenweber, Fusarium-Monographie, pp. 377-378. 1931.
Wollenweber and Reinking, Die Fusarien, p. 92. 1935.

G. pseudopulicaris Wr.
Wollenweber, Fusarium-Monographie, pp. 387-388. 1931.
Wollenweber and Reinking, Die Fusarien, p. 96. 1935.

G. baccata, the perfect stage of *F. lateritium*, has not been found on cereals, or on other hosts, in Canada.

Section Liseola Wr., Sherb., Rg., Johann, & Bailey

Wollenweber, Sherbakoff, Reinking, Johann, & Bailey, J. Agr. Research, 30: 841. 1925.
Wollenweber, Fusarium-Monographie, pp. 388-391. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 96-98. 1935.

The isolates of *Fusarium* from cereal seed that belonged in the section Liseola were classified according to the revision of this section of the genus by Snyder and Hansen (39). In this revision, the description of one species, namely, *F. moniliforme* Sheld., was emended to include *F. moniliforme* var. *anthophilum* (A. Br.) Wr., *F. moniliforme* var. *minus* Wr., *F. moniliforme* var. *subglutinans* Wr. & Rg., *F. lactis* Pir. & Rib., and *F. neoceras* Wr. & Rg.

FUSARIUM MONILIFORME Sheld. emend. Snyder & Hansen (Fig. 40)
Snyder and Hansen, Am. J. Botany, 32: 664. 1945.
Sheldon, Nebraska Agr. Expt. Sta. Ann. Rept. 17: 23-32. 1904.
Wollenweber, Fusarium-Monographie, pp. 391-393. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 98-99. 1935.

Syn. *F. moniliforme* Sheld. var. *anthophilum* (A. Br.) Wr.
Wollenweber, Fusarium-Monographie, pp. 397-399. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 101-102. 1935.

F. moniliforme Sheld. var. *minus* Wr.
Wollenweber, Fusarium-Monographie, p. 397. 1931.
Wollenweber and Reinking, Die Fusarien, p. 102. 1935.

F. moniliforme Sheld. var. *subglutinans* Wr. & Rg.
Wollenweber and Reinking, Phytopath. 15: 163. 1925.
Wollenweber, Fusarium-Monographie, p. 397. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 100-101. 1935.

F. lactis Pir. & Rib.
Wollenweber and Reinking, Die Fusarien, p. 103. 1935.

F. neoceras Wr. & Rg.
Wollenweber and Reinking, Phytopath. 15: 164. 1925.
Wollenweber, Fusarium-Monographie, p. 399. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 103-104. 1935.

Conidia (Fig. 19)

Microconidia, 0–sept. 6.6–11.7 × 2.8–3.4; typically borne in chains.
Macroconidia, 5(3–7)–septate; 3–sept. 29.7–53.0 × 2.9–4.9
5–sept. 46.6–59.4 × 3.6–4.2
7–sept. 59.4–67.8 × 3.8–4.2

Chlamydospores absent. Growth rate 4.6 cm.

Prevalence in Cereal Seed in Canada

The relatively few isolates of *F. moniliforme* obtained during the present investigation indicate that it is seldom seed-borne in wheat, barley, and oats in Canada. In the samples that originated in Eastern Canada, this species was not encountered in wheat or barley and in only four (four kernels) of oats; in the samples from Western Canada it was not encountered in barley and in only one (one kernel) of wheat and in two (three kernels) of oats. *F. moniliforme* was previously reported by Gordon (13) from the occasional farm sample of wheat, barley, and of oats that originated in Manitoba.

Snyder and Hansen (39), in 1945, proposed the name *G. moniliforme* (Sheld.) Snyder & Hansen to denote the perfect stage of *F. moniliforme* Sheld. emend. Snyder & Hansen. The synonym *G. fujikuroi* (Saw.) Wr. has priority, however, as the specific epithet *fujikuroi* dates from 1917, when it was published by Sawada (33) in the binomial *Lisea fujikuroi*. The combination *G. fujikuroi* was made by Wollenweber (47) in 1931, when he transferred *Lisea fujikuroi* Saw. to the genus *Gibberella*.

GIBBERELLA FUJIKUROI (Saw.) Wr.
Wollenweber, Fusarium-Monographie, p. 514. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 99-100. 1935.

Syn. *Lisea fujikuroi* Saw.
Sawada, Ber. naturhist. Ges. Formosa, 31: 131-133. 1917.

G. moniliformis (Sheld.) Wineland
Wineland, J. Agr. Research, 28: 920. 1924.
Wollenweber, Fusarium-Monographie, pp. 393-395. 1931.

G. fujikuroi (Saw.) Wr. var. *subglutinans* Edwards
Edwards, Agr. Gaz. New South Wales, 44: 895-897. 1933.
Wollenweber and Reinking, Die Fusarien, p. 101. 1935.

G. moniliforme (Sheld.) Snyder & Hansen
Snyder and Hansen, Am. J. Botany, 32: 664. 1945.

G. fujikuroi, the perfect stage of *F. moniliforme*, has not been found on cereals or on other hosts in Canada. Perithecia of this fungus may be expected to occur particularly on overwintered stalks of corn (*Zea mays* L.).

Section Elegans Wr.

Wollenweber, Phytopath. 3: 28. 1913.
Wollenweber, Fusarium-Monographie, pp. 400-406. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 104-109. 1935.
Snyder and Hansen, Am. J. Botany, 27: 64-67. 1940.

The majority of the isolates of *Fusarium* that were obtained from cereal seed, and belong in this section of the genus, were identified as *F. oxysporum* Schlecht. emend. Snyder & Hansen. Certain other isolates, however, could be differentiated morphologically as well as culturally from those identified as *F. oxysporum* Schlecht. emend. These isolates were readily identifiable as *F. redolens* Wr. according to the system of classification of Wollenweber and Reinking (50). As indicated by these authors, the macroconidia of *F. redolens* are somewhat wider in the upper third than in the middle and resemble the conidia of *F. solani* (Mart.) App. & Wr. (section Martiella). Sherbakoff (35) pointed out that the color of the substratum of *F. redolens* var. *solani* Sherb., a synonym of *F. redolens*, was "different from all other Fusaria of the Section Elegans". It is now proposed that *F. redolens*, which was included by Snyder and Hansen (37) in the synonymy of *F. oxysporum* Schlecht. emend., be differentiated from that species as a variety, namely, *F. oxysporum* Schlecht. emend. Snyder & Hansen var. *redolens* (Wr.) n. comb.

FUSARIUM OXYSPORUM Schlecht. emend. Snyder & Hansen (Fig. 41)
Snyder and Hansen, Am. J. Botany, 27: 66. 1940.
Wollenweber, Fusarium-Monographie, pp. 416-418. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 117-118. 1935.

Syn. *F. angustum* Sherb.
Sherbakoff, N.Y. Cornell Agr. Expt. Sta. Memoir, 6: 203-205. 1915.
Wollenweber, Fusarium-Monographie, pp. 410-411. 1931.
Wollenweber and Reinking, Die Fusarien, p. 113. 1935.

F. bostrycoides Wr. & Rg.
Wollenweber and Reinking, Phytopath. 15: 166-167. 1925.
Wollenweber, Fusarium-Monographie, p. 406. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 109-110. 1935.

F. bulbigenum Cke. & Mass.
Cooke, Grevillea, 16: 49. 1887.
Wollenweber, Fusarium-Monographie, pp. 411-412. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 113-114. 1935.

F. conglutinans var. *citrinum* Wr.
Wollenweber, Fusarium-Monographie, p. 407. 1931.
Wollenweber and Reinking, Die Fusarien, p. 111. 1935.

F. orthoceras App. & Wr.
Appel and Wollenweber, Grundlagen einer Monographie der Gattung *Fusarium* (Link), pp. 141-156. 1910.
Wollenweber, Fusarium-Monographie, pp. 408-409. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 111-112. 1935.

F. orthoceras App. & Wr. var. *longius* (Sherb.) Wr.
Wollenweber, Ann. Mycol. 15: 23. 1917.
Wollenweber, Fusarium-Monographie, p. 410. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 112-113. 1935.

F. oxysporum Schlecht. var. *meniscoideum* Bugn.
Bugnicourt, Encycl. Mycol. XI: 111-114. 1939.
Wollenweber, Fusarium-Monographie II, p. 196. 1944-45.

F. vasinfectum Atk. f. 2 Wr. & Rg.
Wollenweber and Reinking, Die Fusarien, p. 125. 1935.

F. vasinfectum Atk. var. *lutulatum* (Sherb.) Wr.
Wollenweber, Fusarium-Monographie, p. 424. 1931.
Wollenweber and Reinking, Die Fusarien, p. 125. 1935.

F. vasinfectum Atk. var. *zonatum* (Sherb.) Wr.
Wollenweber, Fusarium-Monographie, p. 424. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 125-126. 1935.

Conidia and Chlamydospores (Fig. 20)

Microconidia, 0–sept. 6.4–10.6 × 2.3–3.2
1–sept. 14.8–19.1 × 2.5–3.4

Macroconidia, 3(3–5)–septate; 3–sept. 25.4–38.2 × 2.5–4.0
4–sept. (occasional) 29.7–34.0 × 3.4–4.2
5–sept. (occasional) 31.8–46.6 × 3.6–4.5

Chlamydospores, terminal and intercalary. Growth rate 4.5 cm.

Prevalence in Cereal Seed in Canada

F. oxysporum was seldom found to be seed-borne in cereals in either Eastern or Western Canada. In the samples from Eastern Canada, this species was encountered in only one (one kernel) of wheat, in seven (10 kernels) of barley,

and in 11 (12 kernels) of oats; in the samples from Western Canada, it was encountered in two (two kernels) of wheat, in three (four kernels) of barley, and in three (three kernels) of oats.

FUSARIUM OXYSPORUM Schlecht. emend. Snyder and Hansen var. REDOLENS Wr. n. comb. (Fig. 42)

Syn. *F. redolens* Wr.
Wollenweber, Phytopath. 3: 29-30. 1913.
Wollenweber, Fusarium-Monographie, pp. 425-426. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 126-127. 1935.

F. redolens Wr. var. *solani* Sherb.
Sherbakoff, N.Y. Cornell Agr. Expt. Sta. Memoir, 6: 205-209. 1915.

F. redolens f. 1 Wr.
Wollenweber, Fusarium-Monographie, p. 426. 1931.
Wollenweber and Reinking, Die Fusarien, p. 127. 1935.

F. oxysporum Schlecht. emend. Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 27: 66. 1940.

Conidia and Chlamydospores (Fig. 21)

Microconidia, 0–sept. 7.4–14.8 × 3.2–4.2
1–sept. 17.0–27.6 × 3.4–4.5

Macroconidia, 3(3–5)–septate; 3–sept. 25.4–38.2 × 4.0–5.1
5–sept. 38.1–44.5 × 5.1–5.7

Chlamydospores, terminal and intercalary. Growth rate 4.5 cm.

Prevalence in Cereal Seed in Canada

As *F. oxysporum* var. *redolens* was isolated from only a few kernels of barley and oats, and not at all from wheat, it is apparently not commonly seed-borne in cereals. This variety of *F. oxysporum* was encountered in four samples (four kernels) of barley and in five (six kernels) of oats that originated in Eastern Canada and in two (two kernels) of barley and two (two kernels) of oats from Western Canada.

Section Martiella Wr.

Wollenweber, Phytopath. 3: 30. 1913.
Wollenweber and Reinking, Die Fusarien, pp. 127-129. 1935.
Snyder and Hansen, Am. J. Botany, 28: 738-740. 1941.

Syn. Section Ventricosum
Wollenweber, Phytopath. 3: 32. 1913.
Wollenweber and Reinking, Die Fusarien, p. 140. 1935.
Snyder and Hansen, Am. J. Botany, 32: 662. 1945.

In line with the revision of the sections Martiella and Ventricosum by Snyder and Hansen (38, 39), the name Ventricosum is considered to be a synonym of Martiella.

The isolates of *Fusarium* that were obtained from cereal seed, and belong in this section of the genus, were identified as *F. solani* (Mart.) App. & Wr. emend. Snyder & Hansen. It is proposed, however, that *F. coeruleum* (Lib.) Sacc. be retained as a species separate from *F. solani* emend. Snyder & Hansen in the section Martiella. According to the revision of this section by Snyder and Hansen (38), *F. coeruleum* and. *F. javanicum* Koord. var. *radicicola* Wr. were considered to be synonyms of *F. solani* f. *radicicola* (Wr.) Snyder & Hansen. As Wollenweber and Reinking (50) attributed field black rot and a jellylike stem-end rot of potato tubers to *F. javanicum* var. *radicicola* but a storage rot of the tubers to *F. coeruleum*, it appears doubtful whether these two fungi as well as the two types of disease caused by them, respectively, are the same. *F. coeruleum* is apparently world wide in distribution (48) and is particularly well known as the cause of a serious storage rot of potato tubers in Great Britain (5), Holland (27), and France (21), whereas *F. javanicum* var. *radicicola* as a pathogen of potato tubers would appear to be chiefly confined to certain areas in the United States (50). Wollenweber and Reinking (50) have pointed out that the macroconidia of *F. coeruleum* differ in shape from all other species, varieties, or forms included by them in the Section Martiella, a characteristic feature of this species which appears to be satisfactory for its separation from *F. solani* emend. Snyder & Hansen. According to McKee (23), *F. coeruleum* "is comparatively stable under normal conditions, and mutations in culture are uncommon".

FUSARIUM SOLANI (Mart.) App. & Wr. emend. Snyder & Hansen (Fig. 43)

Snyder and Hansen, Am. J. Botany, 28: 740. 1941.
Snyder and Hansen, Am. J. Botany, 32: 662. 1945.
Wollenweber and Reinking, Die Fusarien, p. 135. 1935.
Wollenweber, Fusarium-Monographie II, pp. 181-182. 1944-45.

Syn. *F. argillaceum* (Fr.) Sacc.

Saccardo, Syll. Fungorum, 4: 718. 1886.
Wollenweber and Reinking, Die Fusarien, pp. 140-141. 1935.
Snyder and Hansen, Am. J. Botany, 32: 662. 1945.

F. caucasicum Letov

Letov, Material f. Mykol. u. Path. (Leningrad), VIII, 1: 8-10. 1929.
Wollenweber, Fusarium-Monographie II, pp. 187-188. 1944-45.

F. javanicum Koord.

Koorders, Verhandel. K. Akad. Wetenschap., Amsterdam, II, 13: 247-248. 1907.
Wollenweber and Reinking, Die Fusarien, pp. 131-132. 1935.
Wollenweber, Fusarium-Monographie II, p. 178. 1944-45.

F. javanicum Koord. var. *ensiforme* (Wr. & Rg.) Wr.

Wollenweber and Reinking, Die Fusarien, pp. 132-133. 1935.
Wollenweber, Fusarium-Monographie II, p. 180. 1944-45.

F. solani (Mart.) App. & Wr. var. *eumartii* (Carp.) Wr. pr. p.
Wollenweber and Reinking, Die Fusarien, p. 138. 1935.
Wollenweber, Fusarium-Monographie II, pp. 184-185. 1944-45.

F. solani (Mart.) var. *martii* (App. & Wr.) Wr.
Wollenweber and Reinking, Die Fusarien, pp. 136-137. 1935.
Wollenweber, Fusarium-Monographie II, pp. 182-183. 1944-45.

F. solani (Mart.) App. & Wr. var. *minus* Wr.
Wollenweber and Reinking, Die Fusarien, p. 134. 1935.
Wollenweber, Fusarium-Monographie II, p. 186. 1944-45.

F. solani (Mart.) App. & Wr. var. *striatum* (Sherb.) Wr.
Wollenweber and Reinking, Die Fusarien, pp. 135-136. 1935.
Wollenweber, Fusarium-Monographie II, p. 187. 1944-45.

F. solani (Mart.) var. *martii* (App. & Wr.) Wr. f. 1 Wr.
Wollenweber and Reinking, Die Fusarien, p. 137. 1935.
Wollenweber, Fusarium-Monographie II, p. 183. 1944-45.

Conidia and Chlamydospores (Fig. 22)

Conidia, 3(0–5)–septate; 0–sept. 8.5–14.8 × 4.2–4.4
1–sept. 20.1–21.2 × 4.7–5.3
2–sept. (occasional) 29.7 × 4.9
3–sept. 27.6–33.9 × 4.5–5.9.
5–sept. (occasional) 31.8–44.5 × 5.3–6.2

Chlamydospores, terminal and intercalary. Growth rate 3.2 cm.

Prevalence in Cereal Seed in Canada

F. solani would appear to occur rarely in cereal seed in Canada. This species was encountered in only one sample (one kernel) of barley that originated in Eastern Canada and in only one sample (one kernel) of oats from Western Canada.

The perfect stage of *F. solani* emend. Snyder & Hansen is—

HYPOMYCES SOLANI Rke. & Berth. emend. Snyder & Hansen

Reinke and Berthold, Untersuch. a.d. Bot. Lab. d. Univ. Göttingen, Heft 1, 27-39. 1879.
Wollenweber, Fusarium-Monographie, p. 427. 1931.
Wollenweber and Reinking, Die Fusarien, p. 141. 1935.
Snyder and Hansen, Am. J. Botany, 28: 741. 1941.

Syn. *H. haematococcus* (Berk. & Br.) Wr.
Wollenweber and Reinking, Die Fusarien, pp. 139-140. 1935.
Wollenweber, Fusarium-Monographie II, pp. 185-186. 1944-45.

H. haematococcus (Berk. & Br.) var. *breviconus* Wr.
Wollenweber and Reinking, Die Fusarien, pp. 134-135. 1935.
Wollenweber, Fusarium-Monographie II, pp. 186-187. 1944-45.

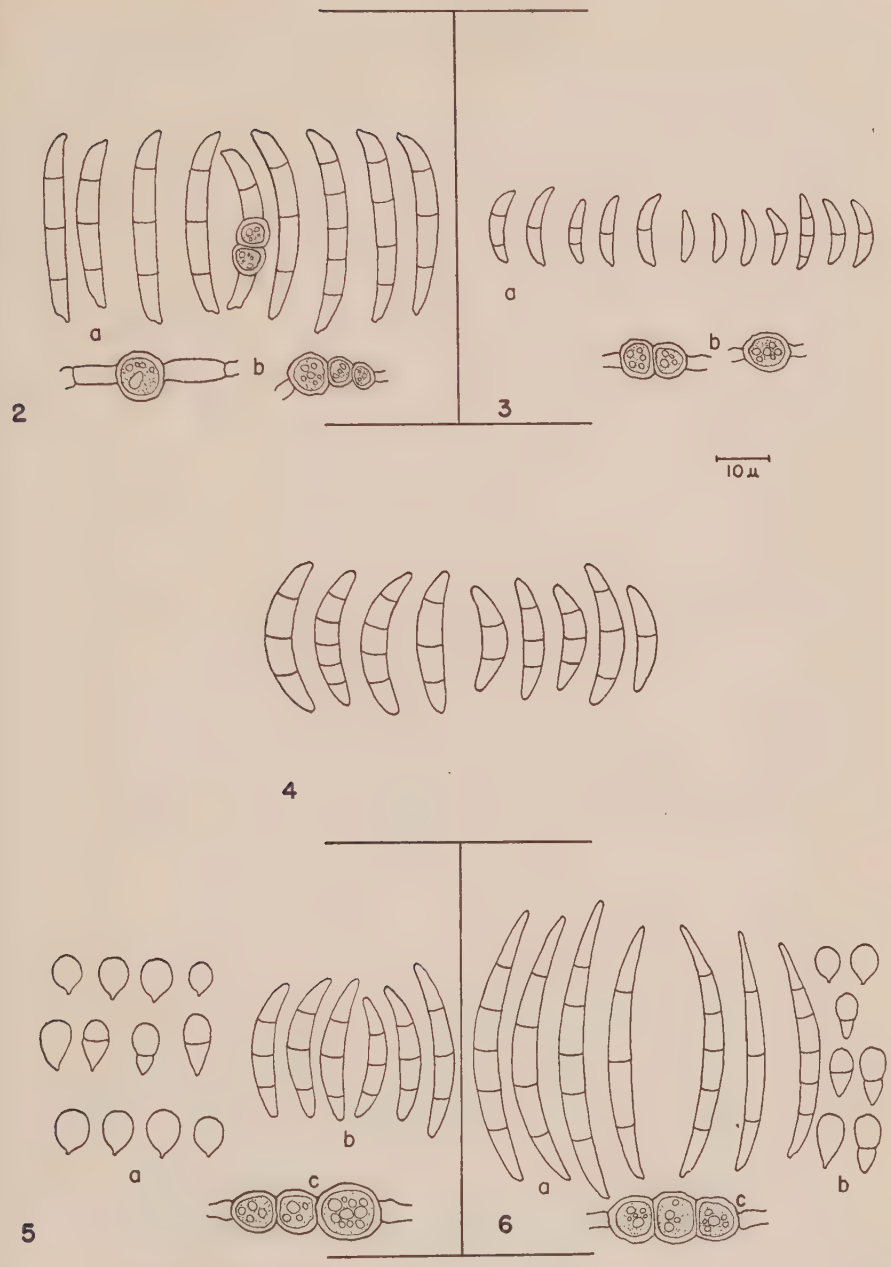


Fig. 2. *F. merismoides: a,* conidia; *b,* intercalary chlamydospores. Fig. 3. *F. dimerum: a,* conidia; *b,* intercalary chlamydospores. Fig. 4. *F. nivale:* conidia. Fig. 5. *F. poae: a,* microconidia; *b,* macroconidia; *c,* intercalary chlamydospores. Fig. 6. *F. sporotrichioides: a,* macroconidia; *b,* microconidia; *c,* intercalary chlamydospores.

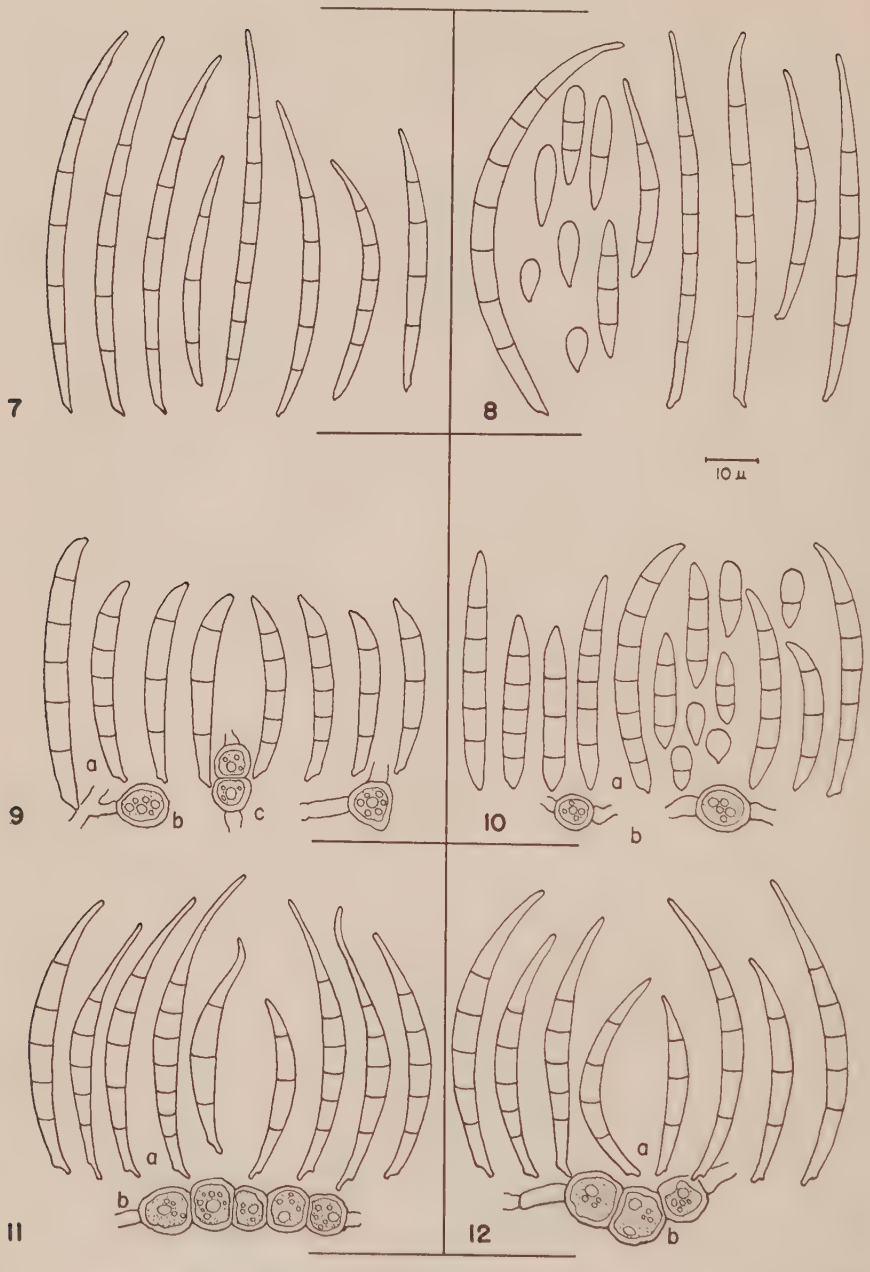
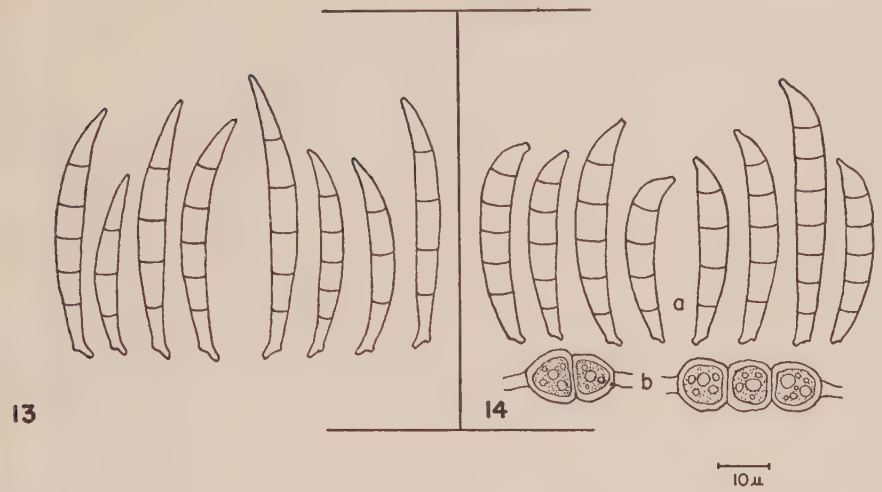
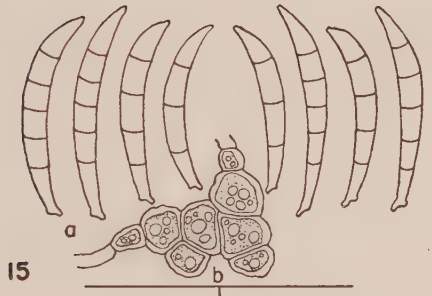


Fig. 7. *F. avenaceum:* conidia. Fig. 8. *F. arthrosporioides:* conidia. Fig. 9. *F. concolor: a*, conidia; *b*, terminal chlamydospore; *c*, intercalary chlamydospores. Fig. 10. *F. semitectum: a*, conidia; *b*, intercalary chlamydospores. Fig. 11. *F. equiseti: a*, conidia; *b*, intercalary chlamydospores. Fig. 12. *F. acuminatum: a*, conidia; *b*, intercalary chlamydospores.





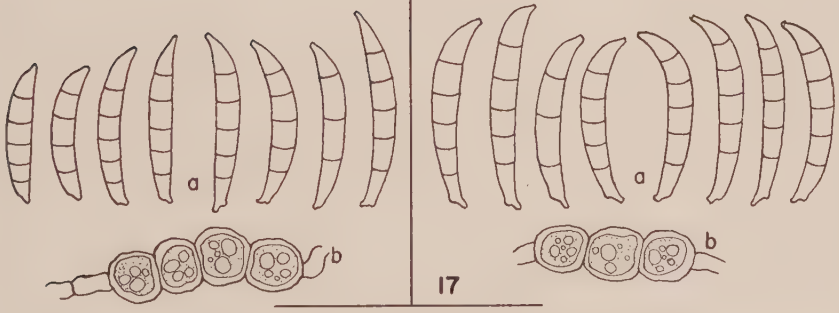


Fig. 13. *F. graminearum:* conidia. Fig. 14. *F. culmorum: a*, conidia; *b*, intercalary chlamydospores. Fig. 15. *F. sambucinum: a*, conidia; *b*, intercalary chlamydospores. Fig. 16. *F. sambucinum* var. *coeruleum: a*, conidia; *b*, intercalary chlamydospores. Fig. 17. *F. sambucinum* f. 6: *a*, conidia; *b*, intercalary chlamydospores.

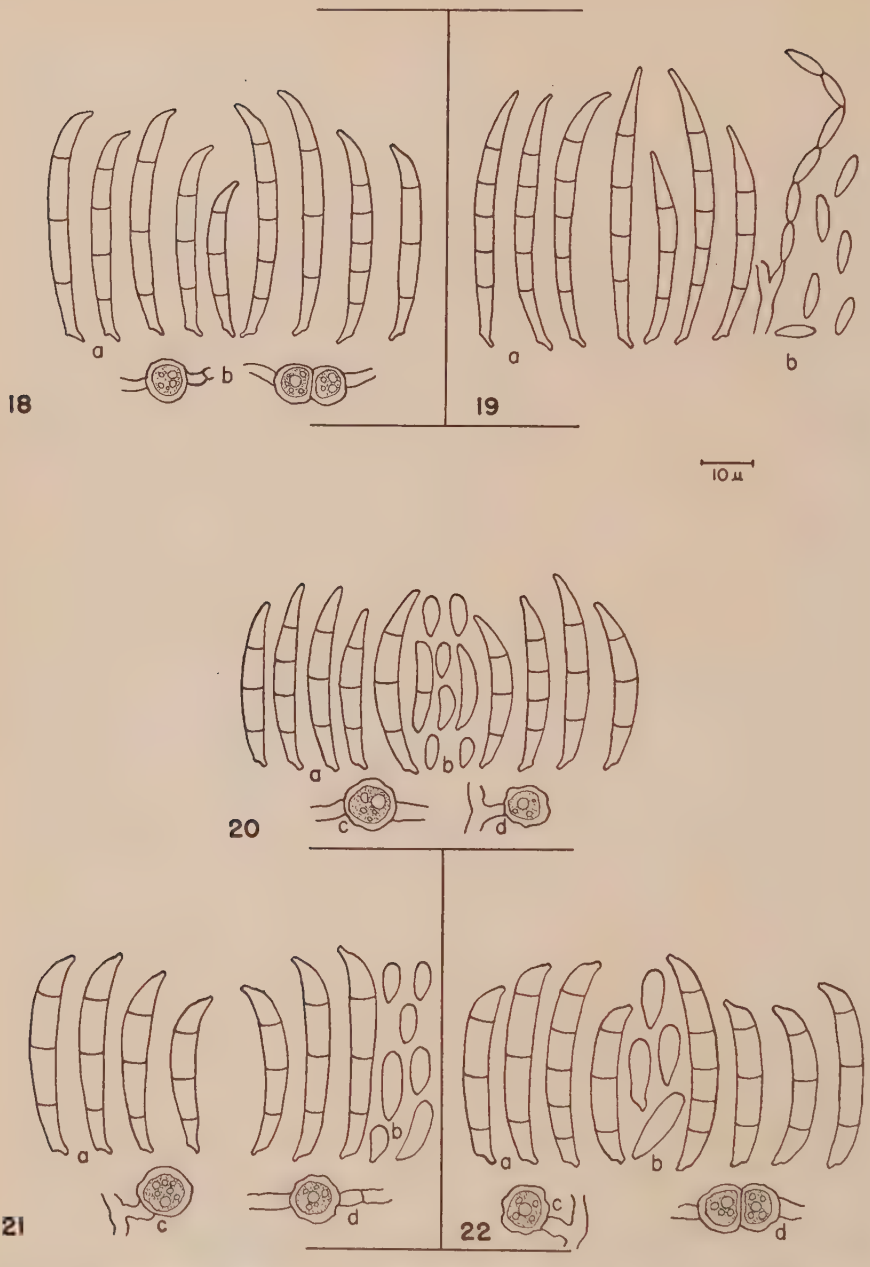


Fig. 18. *F. lateritium: a*, conidia, *b*, intercalary chlamydospores. Fig. 19. *F. moniliforme: a*, macroconidia; *b*, microconidia. Fig. 20. *F. oxysporum: a*, macroconidia; *b*, microconidia; *c*, intercalary chlamydospore; *d*, terminal chlamydospore. Fig. 21. *F. oxysporum* var. *redolens: a*, macroconidia; *b*, microconidia; *c*, terminal chlamydospore; *d*, intercalary chlamydospore. Fig. 22. *F. solani: a*, macroconidia; *b*, microconidia; *c*, terminal chlamydospore; *d*, intercalary chlamydospores.

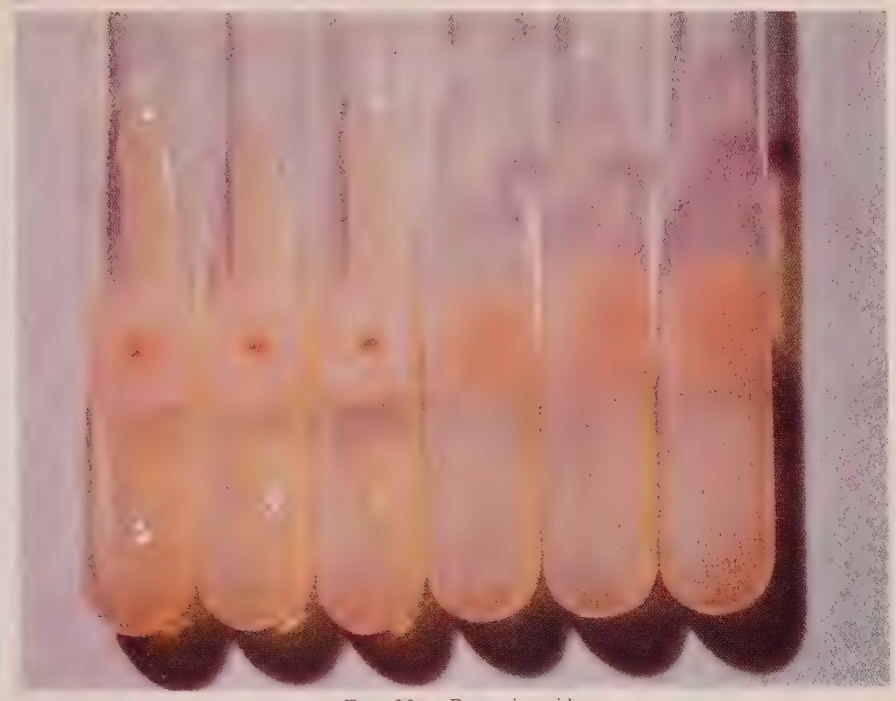


FIG. 23. *F. merismoides.*

FIG. 24. *F. dimerum.*

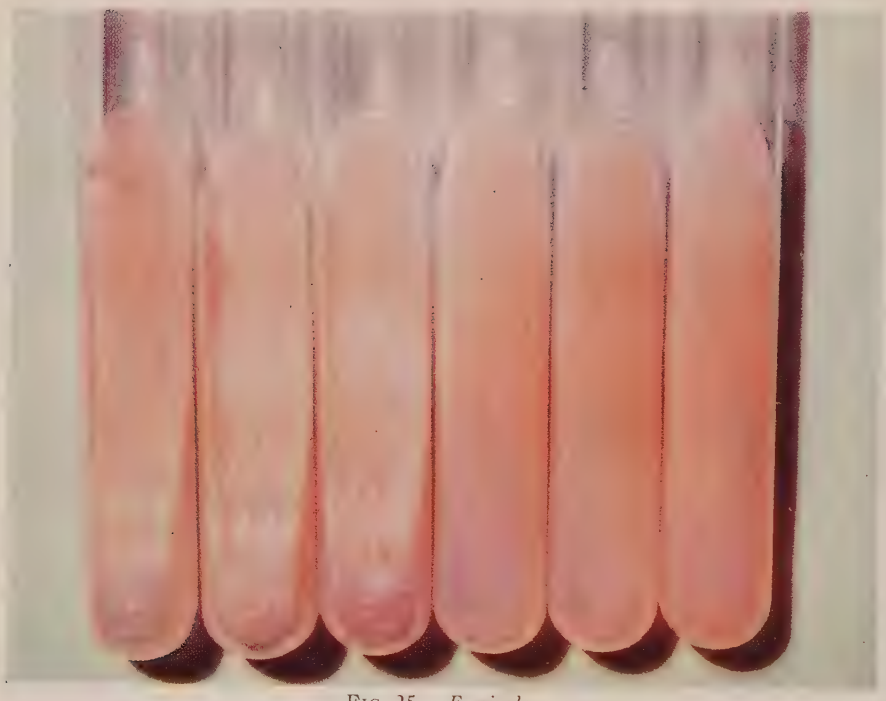


FIG. 25. *F. nivale.*

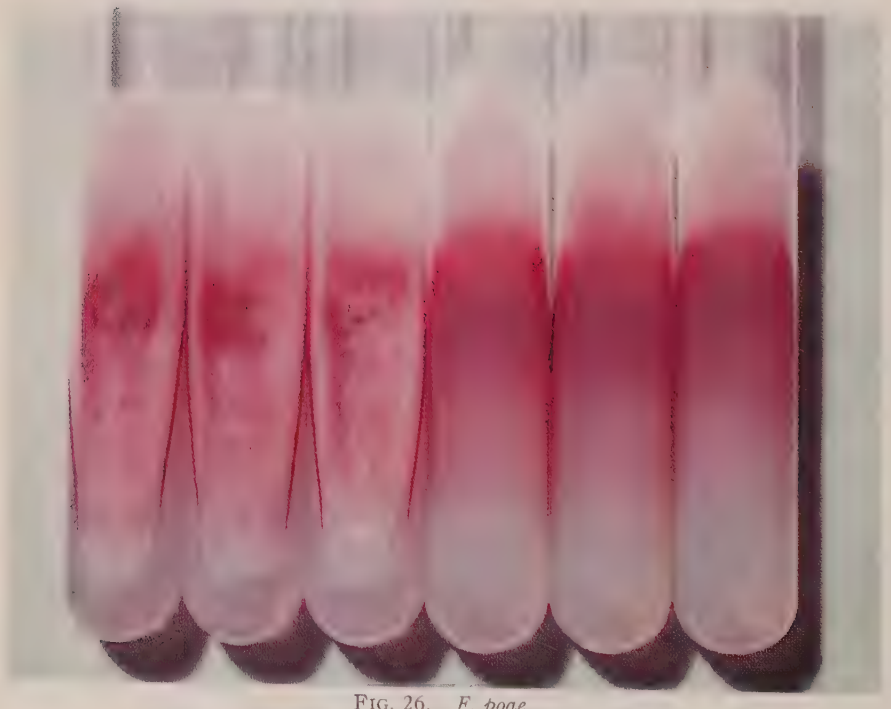


FIG. 26. *F. poae.*

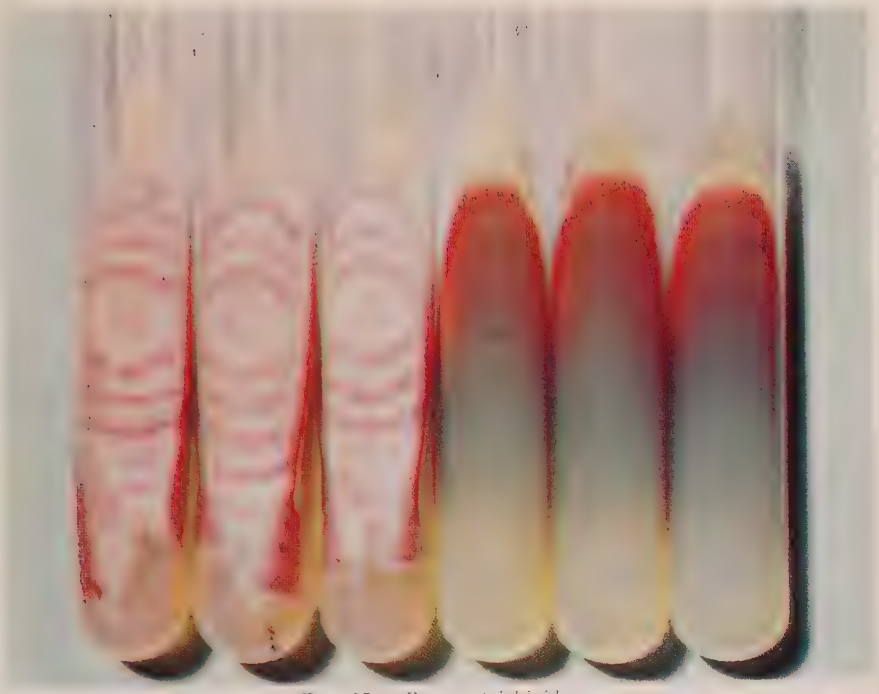


FIG. 27. *F. sporotrichioides.*

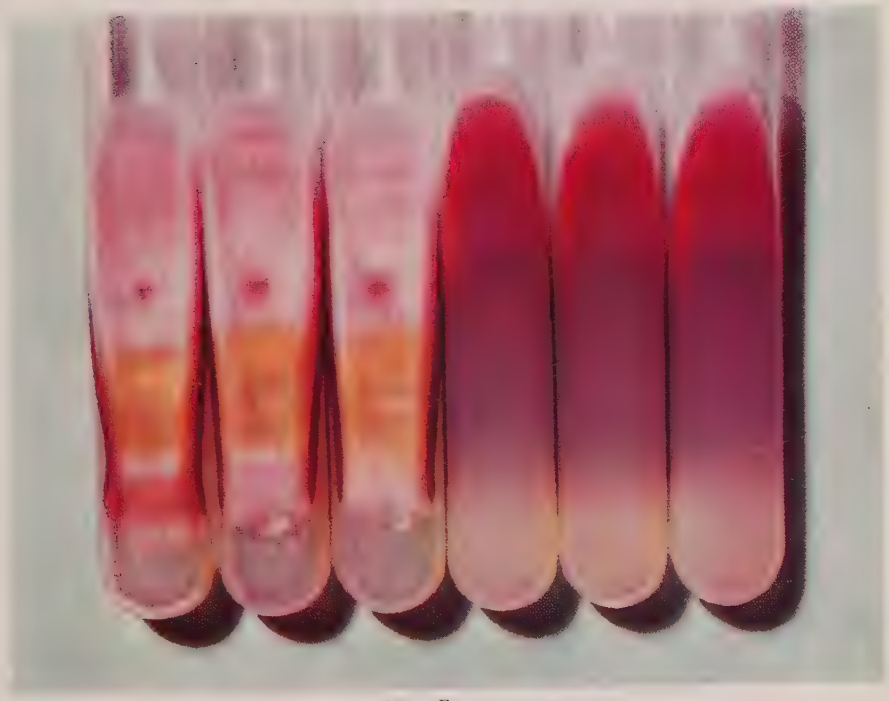


FIG. 28. *F. avenaceum.*

FIG. 29. *F. arthrosporioides.*

FIG. 30. *F. concolor.*

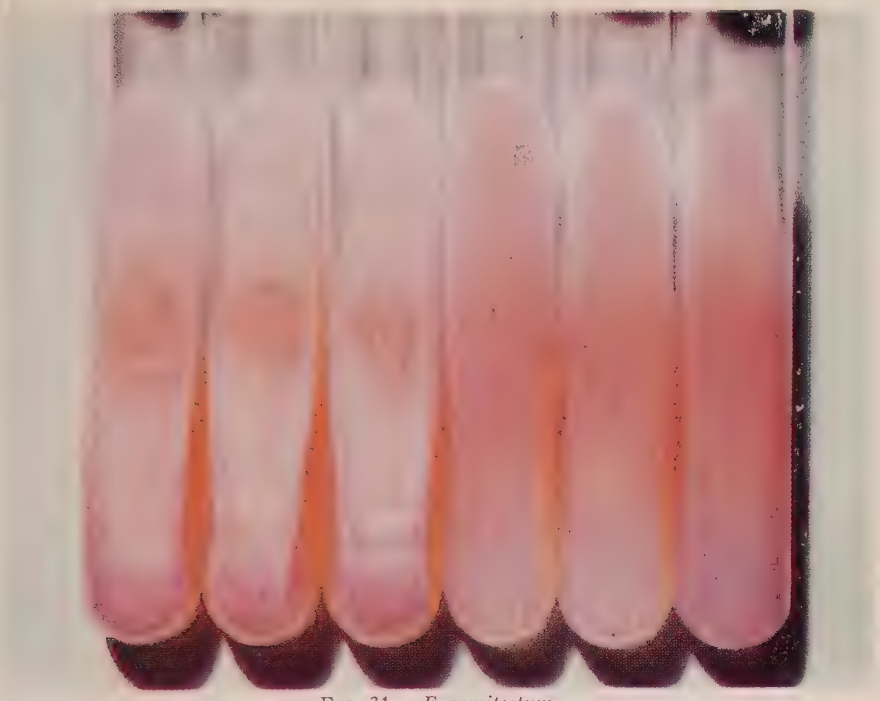


FIG. 31. *F. semitectum.*

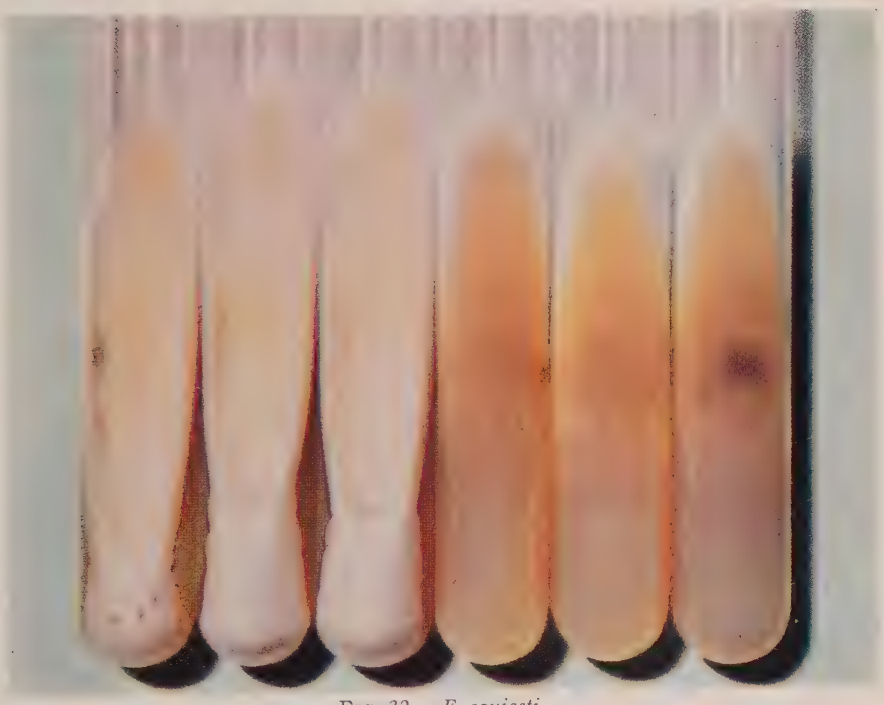


FIG. 32. *F. equiseti.*

FIG. 33. *F. acuminatum.*

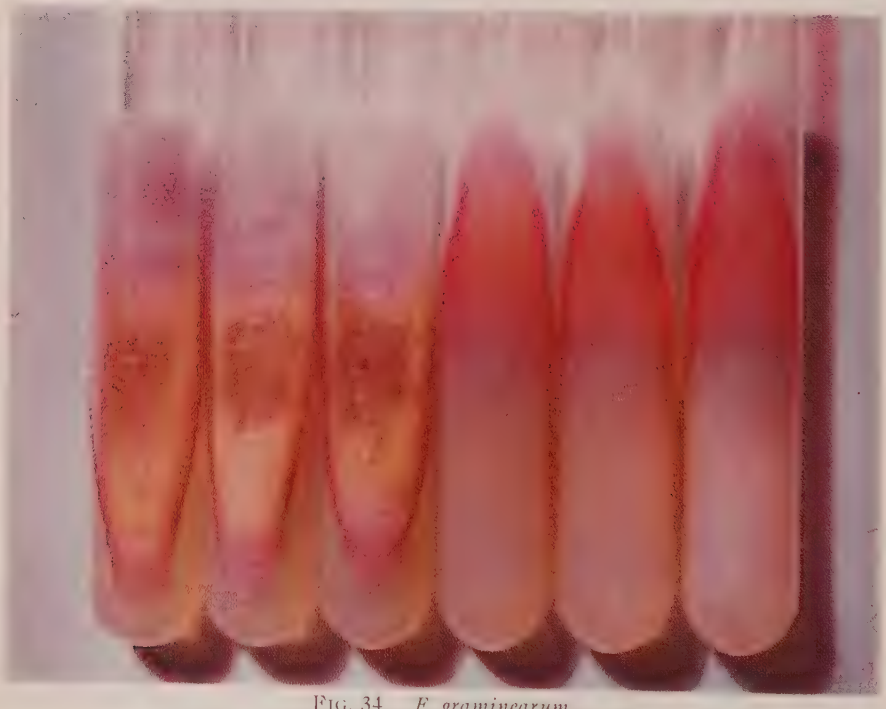


FIG. 34. *F. graminearum.*

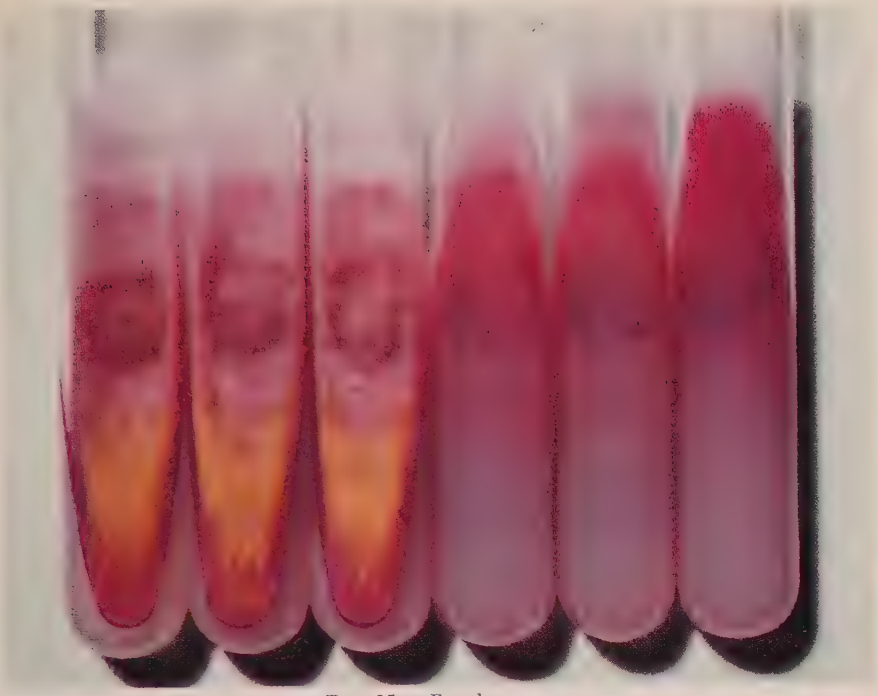


FIG. 35. *F. culmorum.*

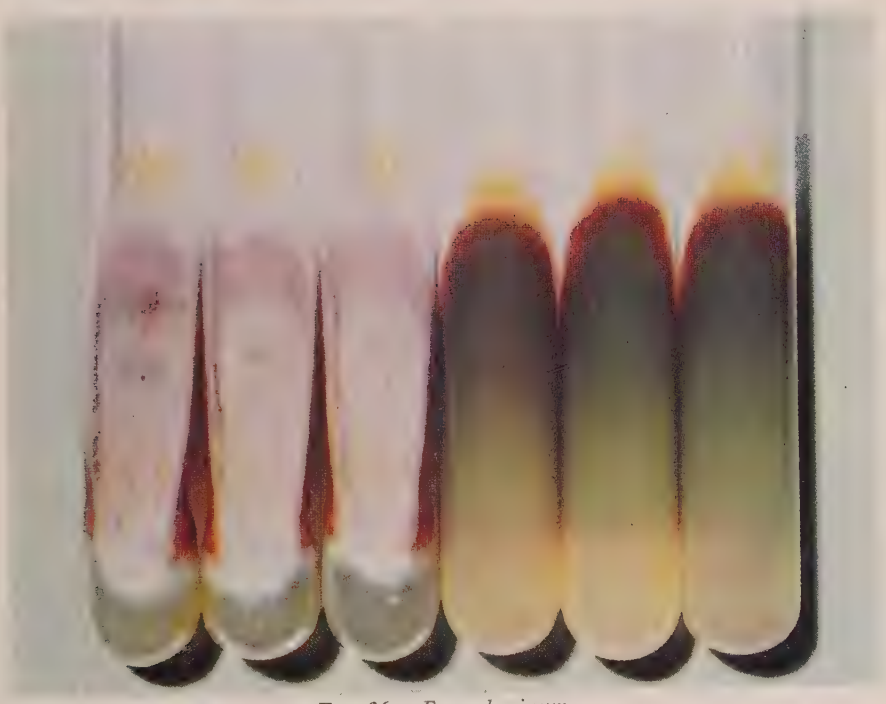


FIG. 36. *F. sambucinum.*

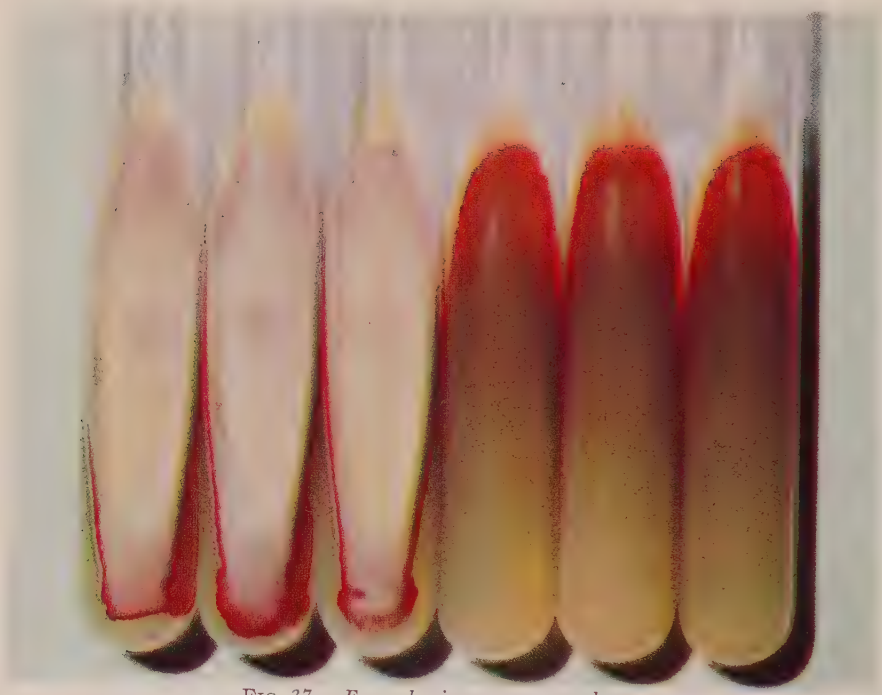


FIG. 37. *F. sambucinum* var. *coeruleum*.

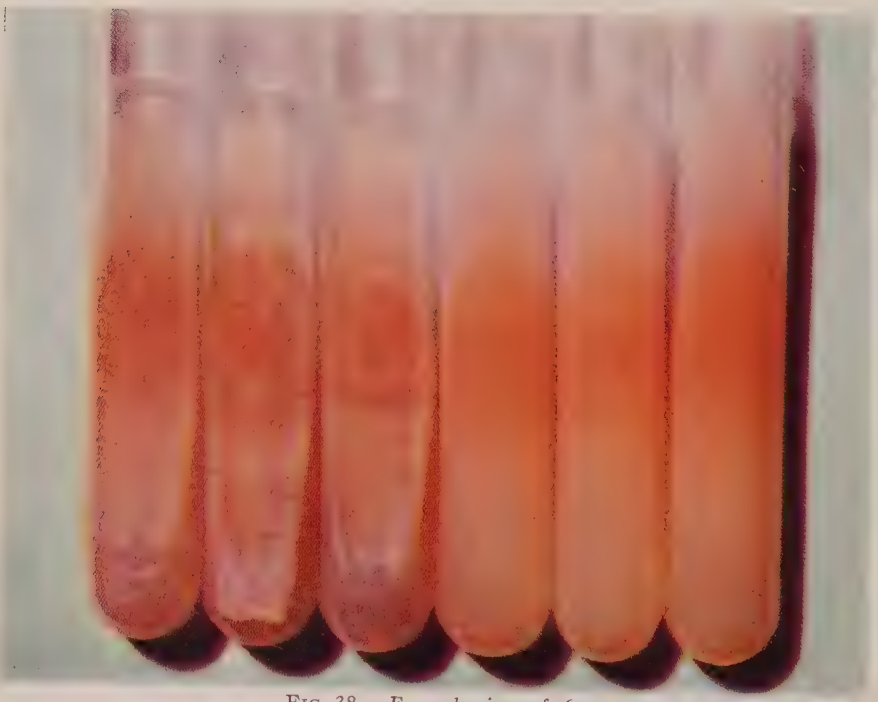


FIG. 38. *F. sambucinum* f. 6.

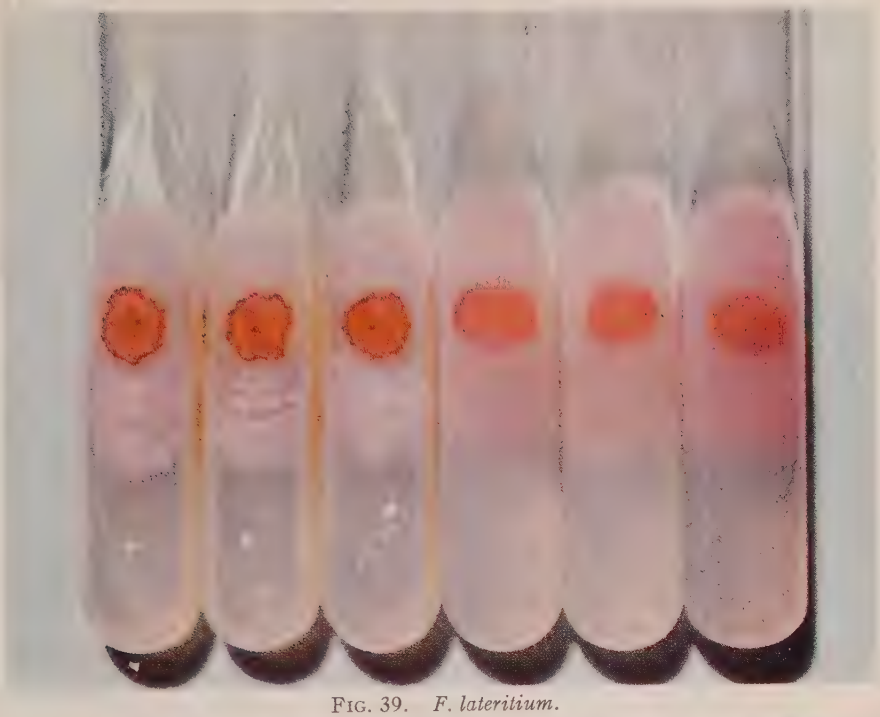


FIG. 39. *F. lateritium.*

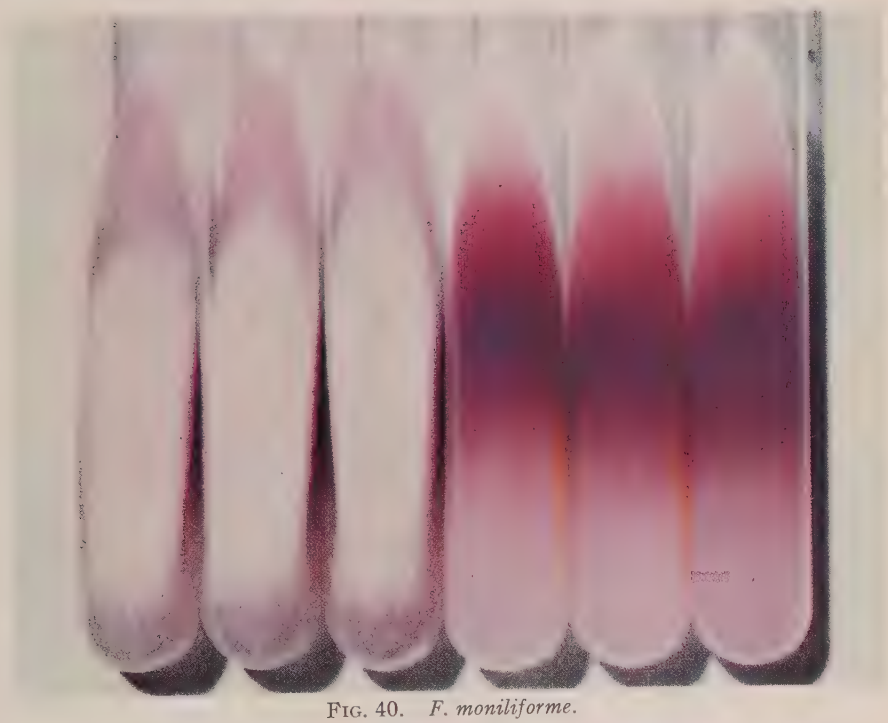


FIG. 40. *F. moniliforme.*

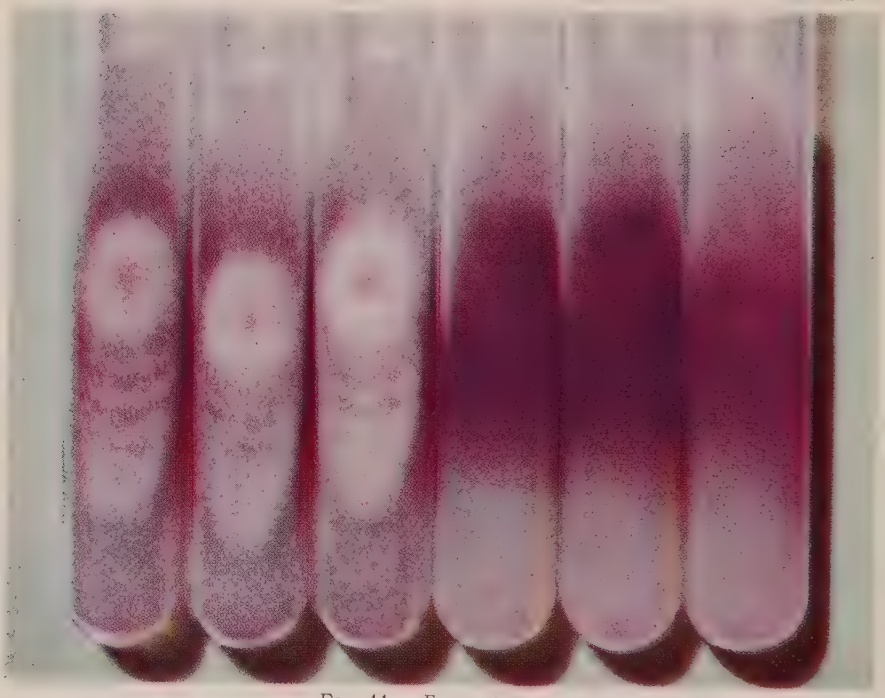


FIG. 41. *F. oxysporum.*

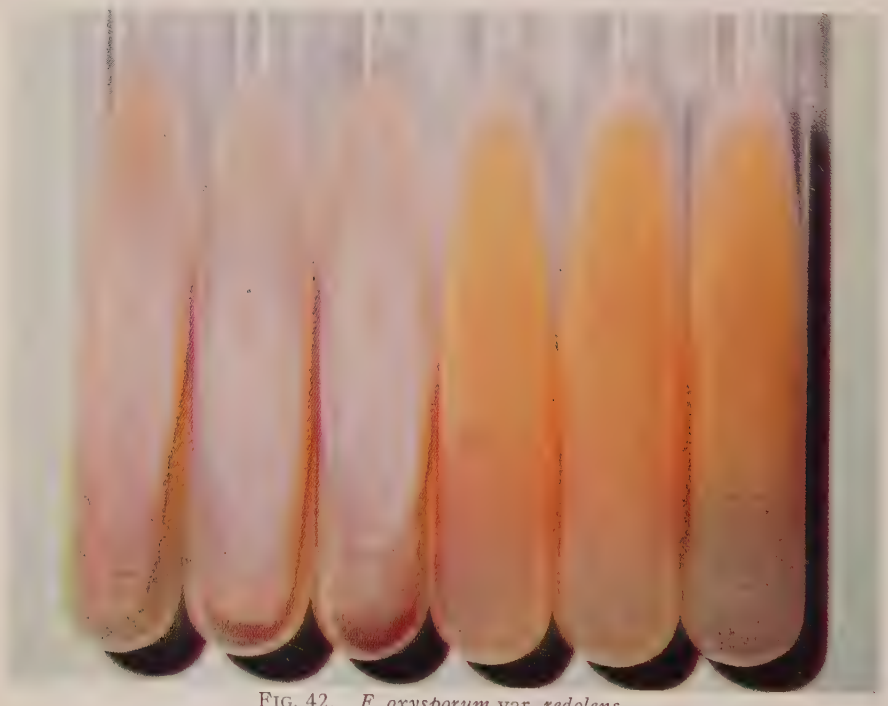


FIG. 42. *F. oxysporum* var. *redolens.*

FIG. 43. *F. solani.*

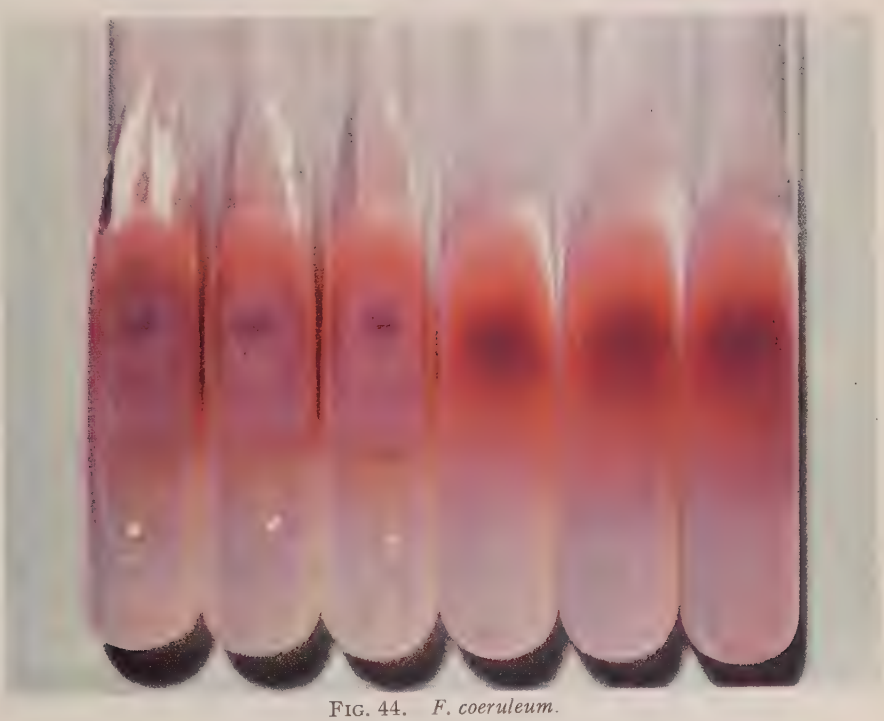


FIG. 44. *F. coeruleum.*

H. haematococcus (Berk. & Br.) var. *cancri* (Rutg.) Wr.
Wollenweber and Reinking, Die Fusarien, p. 136. 1935.
Wollenweber, Fusarium-Monographie II, p. 187. 1944-45.

H. ipomoeae (Hals.) Wr.
Wollenweber and Reinking, Die Fusarien, p. 132. 1935.
Wollenweber, Fusarium-Monographie II, pp. 178-179. 1944-45.

H. ipomoeae (Hals.) var. *major* Wr.
Wollenweber and Reinking, Die Fusarien, p. 133. 1935.
Wollenweber, Fusarium-Monographie II, p. 181. 1944-45.

H. ipomoeae (Hals.) f. 1 Wr.
Wollenweber and Reinking, Die Fusarien, p. 131. 1935.
Wollenweber, Fusarium-Monographie II, p. 180. 1944-45.

Perithecia of *H. solani* have not been collected on cereals or other hosts in Canada.

FUSARIUM COERULEUM (Lib.) Sacc. (Fig. 44)
Saccardo, Syll. Fungorum, 4: 705. 1886.
Wollenweber and Reinking, Die Fusarien, p. 134. 1935.
Wollenweber, Fusarium-Monographie II, pp. 176-178. 1944-45.

Syn. *F. solani* (Mart.) App. & Wr. f. *radicicola* (Wr.) Snyder & Hansen pr. p.
Snyder and Hansen, Am. J. Botany, 28: 740. 1941.

F. coeruleum has not been isolated from cereals in Canada but this species is a common cause of storage rot of potato tubers.

The perfect stage of *F. coeruleum* is:—

HYPOMYCES ASCLEPIADIS Zerova
Zerova, J. Botan. Inst. Acad. Sci., Ukraine, 11: 101-104. 1937.

The perfect stage of *F. coeruleum* was reported by Zerova (51) in 1937 to be a new species of *Hypomyces*, namely, *H. asclepiadis*. An isolate of *F. coeruleum* that had been obtained from decayed, underground stem parts of *Asclepias cornuti* Decaisne in 1931 formed perithecia of *H. asclepiadis* when grown on Leonian's medium.

Discussion

As the samples of cereal seed that were examined microbiologically during this investigation were obtained from many widely separated localities in Canada, the data presented in Table I indicate that *Fusarium* is seed-borne by wheat, barley, and oats rather generally. It will be apparent as well from these data that *Fusarium* is more likely to be seed-borne by oats and barley than by wheat in both Eastern and Western Canada, and that the seed of each of these three crops is more likely to be harboring *Fusarium* if it is produced in Eastern Canada. The presence of the hull on the seed of oats and barley probably accounts for the greater infestation of the seed of these two crops by *Fusarium*. According to the conclusions reached by Hyde (18) from

a study of fungal infestation of wheat seed produced under different environmental conditions, it would appear that the more common occurrence of seed-borne *Fusarium* in wheat, barley, and oats in Eastern Canada than in Western Canada may be partly attributed to the more humid atmospheric conditions prevailing there during the crop period.

The results obtained from the identification of the isolates of *Fusarium* from cereal seed show that a considerable number of species and varieties may be seed-borne by wheat, barley, and oats and that the majority of these species and varieties of *Fusarium* may be seed-borne in both Eastern and Western Canada. Of the total of 16 species and varieties of *Fusarium* that was isolated, six species, namely, *F. poae*, *F. avenaceum*, *F. acuminatum*, *F. equiseti*, *F. culmorum*, and *F. oxysporum* were seed-borne by wheat, barley, and oats in both Eastern and Western Canada. Seven additional species and varieties, namely, *F. sporotrichioides*, *F. graminearum*, *F. sambucinum*, *F. sambucinum* var. *coeruleum*, *F. moniliforme*, *F. oxysporum* var. *redolens*, and *F. solani*, were seed-borne in both Eastern and Western Canada but not by all three crops in both of these geographic areas. Three species that were rarely isolated, namely, *F. arthrosporioides*, *F. semitectum*, and *F. lateritium*, were seed-borne only in Eastern Canada, by wheat, barley, and oats, respectively.

In order to appraise the possible importance that these species and varieties of *Fusarium* may have in the production of disease in cereals, particularly seedling blight and root rot, consideration must be given to their pathogenicity as well as to their prevalence in cereal seed. The species and varieties of *Fusarium* that were isolated may be classified into three groups on the basis of their pathogenicity to cereals, as reported by Bennett (2), Gordon and Sprague (16), Johnston and Greaney (20), McKay (22), Oswald (28), Simmonds and Ledingham (36), Sprague (40), and others. In the first of these three groups, consisting only of species widely recognized as being primary pathogens of cereals, *F. graminearum* and *F. culmorum* may be included. These two species would not appear to be commonly seed-borne by cereals in Canada, as less than 0.02% of the cereal seed examined harbored either one of them. In the second group, consisting of species which have usually been found to be only moderately or even weakly pathogenic to cereals, may be included *F. poae*, *F. sporotrichioides*, *F. avenaceum*, *F. acuminatum*, *F. equiseti*, and *F. oxysporum*. Two species in this group, namely, *F. poae* and *F. avenaceum*, are evidently more commonly seed-borne than any of the other species that were isolated. Approximately 1.5% of the cereal seed that was examined harbored *F. poae*, and approximately 0.25% yielded *F. avenaceum*. Although these two species appear to be of minor importance to cereals in Canada from the standpoint of their ability to cause seedling blight and root rot, *F. avenaceum* particularly may cause an appreciable amount of head blight under conditions of relatively high humidity and temperature. Although *F. acuminatum*, *F. equiseti*, and *F. oxysporum* are rather frequently associated with root rot of cereals when approaching maturity, and are associated occasionally with head blight of cereals in Canada, they do not appear to be commonly

seed-borne, as each of these species was isolated from relatively few seeds. In the third group, consisting of species and varieties that are known to exhibit little, or no pathogenicity to wheat, barley, and oats, are included *F. arthrosporioides*, *F. semitectum*, *F. sambucinum*, *F. sambucinum* var. *coeruleum*, *F. lateritium*, *F. moniliforme*, *F. oxysporum* var. *redolens*, and *F. solani*. With the exception of *F. oxysporum* var. *redolens*, which is frequently associated with root rot of cereals, apparently as a saprophyte, the species and varieties of *Fusarium* included in this group are seldom associated with disease in wheat, barley, and oats in Canada and evidently they are seed-borne only occasionally by these crops. To this third group may be added *F. concolor* and *F. sambucinum* f. 6, which were reported by Gordon (13) to be seed-borne occasionally in Manitoba by barley and wheat, respectively.

With the exception of *F. nivale*, all the more important *Fusarium* pathogens of wheat, barley, and oats were found to be seed-borne by these crops. As only a minor percentage of the seed that was examined was found to be harboring *Fusarium*, it would appear that *Fusarium*-infested seed is not likely to create a serious cereal disease problem, generally, in Canada.

As the classification of most fungi has been based on the characters that they exhibit in nature, the suggestion made by Miller (26) that the descriptions of *Fusarium* species should be based on the characters of the wild types is appropriate. A feature of *Fusarium* identification is that the characters of the wild types must be determined largely from their growth in culture, for only in culture may there be observed all the characters that are required for their identification. The presence of *Fusarium* in nature probably is most often detected when isolations are made from plant parts, etc., although occasionally conidial masses may be encountered. As the wild types of all species, varieties, and forms of *Fusarium* grow readily in culture, it is possible to determine their morphological and cultural characteristics under controlled environmental conditions. Some wild types of *Fusarium* sporulate normally at once in culture; others at first may produce only microconidia; and still others may not sporulate normally for several days, or even weeks, although they may at first produce a few conidia that are seldom of value in their identification. Owing to mutant strains that may soon arise in wild-type cultures, it cannot be taken for granted that the conidia produced in abundance after cultures are a few days old have been formed by the wild type. Whether or not the conidia to be found in a wild-type culture have been formed by it may be ascertained readily by culturing several of these conidia singly. If these monoconidial cultures are apparently identical with the wild type, the conidia from which they developed were undoubtedly formed by it, for conidia taken from cultures of mutant strains of *Fusarium* have not been found to reproduce the wild type, although mutant strains of *Fusarium* that have arisen in cultures of previous mutant strains have been occasionally found to revert to their parent mutant types. If mutant strains of *Fusarium* that arise from wild types do revert to them, such reversions are apparently rare.

The wild types of the species and varieties of *Fusarium* that were isolated from cereal seed and kept in culture under laboratory conditions were found to mutate readily, so that the usual method of repeated subculturing of fungi by mass transfers was not suitable for the retention of the wild types. Apparently they may be retained indefinitely in culture, on potato sucrose agar, for example, if during each consecutive transfer of them, several monoconidial or single hyphal tip cultures are made and the wild-type cultures selected out each time. When wild types were transferred at monthly intervals by making 10 monoconidial cultures of each, usually at least five of each lot of 10 cultures would be the wild type. The longer the interval between transfers of the wild types by the monospore transfer method, the greater was the proportion of mutant to wild-type cultures.

The sterile soil method developed by Miller (24) and employed successfully by Cormack (8), was found to be suitable for the retention of the wild types of *Fusarium* that were obtained from cereal seed. It should be emphasized, however, that in order to avoid transferring mutants to the sterile soil tubes along with the wild types, transfers should be made from young cultures of the wild types, preferably not more than four days old.

The advantages to be gained by the use of wild types of *Fusarium* in pathogenicity tests are shown by the results obtained by Eide (10), Miller (24), Oswald (28), and Cormack (8). Usually, but not invariably (42), cultural mutants are found to be less pathogenic than wild types. If the wild types have abundant, high, aerial mycelium in culture, it would appear that the cultural mutants which resemble the wild types in this respect are generally more pathogenic than other mutants with little or no aerial mycelium.

The range of variation in the characters of the wild types of species, varieties, and forms of *Fusarium* belonging in the sections Roseum, Arthrosporiella, Gibbosum, and Discolor was not observed to result in overlapping to an extent that would justify combining these four sections into one species, namely *F. roseum*, as proposed by Snyder and Hansen (39). The adoption of their revision (37, 38, 39), with minor changes, of the sections Lateritium, Liseola, Martiella, and Elegans, appeared to be advisable for a more practical classification of the wild types of *Fusarium* belonging in these sections. Relatively minor changes within sections Eupionnotes, Arachnites, and Sporotrichiella, compared with the revision of these sections by Snyder and Hansen (39), were required to permit identification with certainty of the wild types of *Fusarium* from cereal seed.

Although a single system of classification of the species, varieties, and forms of the genus *Fusarium* based on the characters of the wild types is to be desired, the attainment of this goal cannot be expected until our knowledge of the wild types that exist in various regions of the world is more complete. Even then, as pointed out by Snyder and Hansen (39), taxonomic systems are not likely to remain fixed, for new information is constantly being added that will influence our classification of "biological units". This is particularly true with respect to the genus *Fusarium*, the wild types of which are worldwide in

distribution as saprophytes and parasites and are mutable in nature as well as in culture. It appears likely that when the wild types of *Fusarium* have been ascertained in various regions, the variability observed in their characters will require the adoption of a simplified system of classification along the lines proposed by Snyder and Hansen (37, 38, 39). A system of classification of this nature, however, may be found to be too general to be of value in differentiating the wild types of *Fusarium* in any one region. If so, a supplementary regional classification may be required.

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CARBON AND OXIDATION–REDUCTION BALANCES AND THE ESTIMATION OF FERMENTATION EFFICIENCIES OF AEROBIC FERMENTATIONS[1]

By P. Shu and J. A. Thorn

Abstract

A method for preparing carbon and oxidation–reduction balances of aerobic fermentations is described. In spite of the complexity of the microbial cell, relatively few and simple analyses of the material are necessary for the purpose of constructing the balances. To test the method, balances were made on citric acid and ustilagic acid fermentations. Excellent results were obtained. Based on the principle of material balance, a number of formulae for calculating the fermentation efficiency and respiratory loss have been established for fermentations involving substrates and products of varied oxidation–reduction status. Limitations of the method and formulae are also discussed. Results obtained by the described methods and equations are of value in correlating the various processes occurring in an aerobic fermentation and in providing an over-all picture of the fermentation.

Introduction

In the study of microbial metabolism, the construction of carbon balance sheets is generally considered to be of value. Raistrick and his associates (11) found carbon balance data very useful in their search for new fungal metabolic products and for the classification of fungi. In their studies the surface culture method was used, and fermentations were carried out in closed flasks with intermittent passage of oxygen-containing gas. Volatile and gaseous products were recovered from the exhaust gas. Recently, Whitaker (18) investigated the metabolic products of wood-rotting fungi with a similar technique. From the carbon balance data of a citric acid fermentation of glucose by *Aspergillus niger*, Wells *et al.* (17) concluded that the acid formation did not occur through a process involving the breakdown of glucose as in alcoholic fermentation. Koepsell (6) also used carbon balance data, obtained at different stages of a fermentation, to support the mechanism of gluconate oxidation by resting cells of *Pseudomonas fluorescens*. However, his attempt to obtain an oxidation–reduction balance was not successful.

Oxidation–reduction balance data are useful, not only in (*a*) checking a carbon balance and in (*b*) aiding in the identification of unknown products by revealing their oxidation–reduction status, but are also useful in the estimation of the theoretical yield of a fermentation. The significance of and the principles involved in the construction of an oxidation–reduction balance for anaerobic fermentations were fully described by Johnson *et al.* (5).

For aerobic fermentations, however, the construction of oxidation–reduction balances and the calculation of theoretical yields are complicated by the formation of relatively large amounts of complex cell materials and the utilization of oxygen as substrate. In the present study, a method was

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designed to overcome these difficulties, and general formulae were derived to estimate fermentation efficiencies and respiratory losses. Data for two different types of fermentations, i.e. citric acid and ustilagic acid fermentations, are presented as illustrations.

Methods and Apparatus

Apparatus and Procedure

Fig. 1 shows that the apparatus consists of three major systems, namely a fermentor attached to a carbon dioxide absorber; an oxygen reservoir and

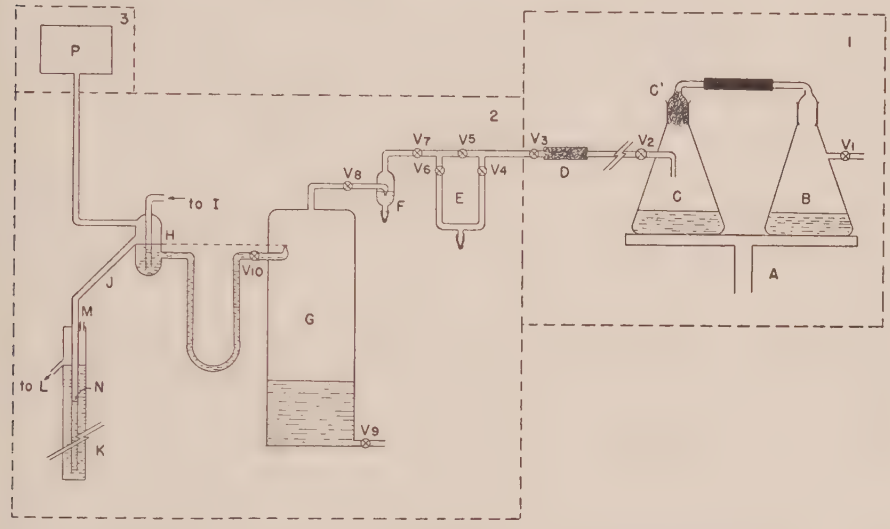

FIG. 1. An apparatus for carbon and O/R balances of aerobic fermentations.

1. Fermentor and carbon dioxide absorber system.
   - *A*. Rotary shaker
   - *B*. Carbon dioxide absorber (500 ml. Erlenmeyer) containing sodium hydroxide
   - *C*. Fermentor flask (500 ml. Erlenmeyer) containing medium
   - *C'*. Cotton plug
   - *D*. Cotton filter
2. Oxygen reservoir, water supply system.
   - *E*. Differential manometer
   - *F*. Water sealed check valve
   - *G*. Oxygen reservoir
   - *H*. Water reservoir
   - *I*. Water tap
   - *J*. Wide connecting tube
   - *K*. Water seal device
   - *L*. Drainage
   - *M*. Vent
   - *N*. Water level inside the tube indicating the pressure differential between atmosphere and water reservoir
   - V_1—V_{10}. Stopcocks
3. Manostat system.
   - *P*. Electronically controlled manostat.

water supply; and a manostat. The entire apparatus is assembled in an incubation room kept at 28°C.

The fermentor flask *C*, containing a known amount of synthetic medium, is sterilized together with the cotton filter *D* and the rubber tubing connecting them. Since the passage between fermentor *C* and absorber *B* is loosely plugged with cotton plug *C'*, the absorber *B* need not be sterilized. After inoculation, the fermentor is connected to the absorber which contains a known volume of 4 *N* sodium hydroxide (the amount should be sufficient to absorb all the carbon dioxide produced by the fermentation). Using V_1 and V_3 the flasks are repeatedly evacuated and filled with oxygen-containing gas. At the end of this process, the pressure inside the flasks is made slightly higher than that under which the fermentation is to be conducted and stopcocks V_1 and V_3 are closed. The flasks are then allowed to reach temperature equilibrium.

The oxygen reservoir *G* is first filled with water through V_9 with V_4, V_6, V_{10} closed and V_5, V_7, V_8 open. The water in the reservoir is then displaced by oxygen through the open end of the differential manometer *E* with V_9 open. After the water is completely displaced, V_5 and V_9 are closed with the reservoir kept at a pressure slightly higher than that of the water reservoir *H* (which is kept at constant pressure with manostat *P*). The open end of V_3 of the fermentor–absorber unit is then connected to the open end of manometer *E*. After temperature equilibrium is reached, V_{10} is turned to the open position and the gas pressure in reservoir *G* is adjusted to that of the water reservoir *H* by releasing the excess pressure through V_9. The stopcocks V_3, V_4, and V_6 are then opened, and the excess gas pressure in the fermentor and absorber is released through V_1 until the differential manometer indicates that the pressures in *G* and *C*, *B* are in equilibrium. V_4 and V_6 are then closed and V_5 opened. Thus, pressure equilibrium in the entire system *C*, *B*, *G*, *H* is obtained. The fermentation is then begun by placing the fermentor–absorber unit on a rotary shaker running at 210 r.p.m. with a 1 in. eccentricity.

In the course of a fermentation, the oxygen uptake of the organism offsets the pressure equilibrium in the closed system. An equivalent amount of water is then drawn into reservoir *G* from water reservoir *H* through V_{10}, resulting in the displacement of oxygen from *G* to fermentor *C* through the water seal *F*. The water level in chamber *H* is kept constant by a flow of water through *H* and a water seal tube *K*. The tube *J* connecting *H* and *K* must be sufficiently wide so that the overflow from *H* will not fill up the entire tubing.

In spite of the fact that the apparatus can supply oxygen continuously to the fermentor, the best fermentations have been obtained with intermittent replenishment of oxygen by periodically closing V_2 and clamping the rubber tubing connecting flasks *B* and *C* daily for about 16 hr. periods. (This was true at least for citric and ustilagic acid fermentations.)

After the fermentation is completed, V_2 and V_{10} are closed and the fermentor–absorber unit is disconnected from the oxygen reservoir at V_3. If the

final culture broth requires acidification for complete release of carbon dioxide, a small amount of phosphoric acid is introduced quickly to the precooled fermentor through V_2, and the latter closed immediately. Afterwards, the fermentor–absorber unit is again placed on the shaker for about two hours. The contents of the fermentor are then separated into cells and products for chemical analyses. A sample is taken from absorber *B* for carbon dioxide determination. The volume of water accumulated in the oxygen reservoir *G* is measured, and the amount of oxygen uptake is calculated with temperature and pressure corrections.

Analyses of Fermentation Products

For the convenience of analyses, the fermentation products are divided into (*a*) metabolic products, and (*b*) cell material. The nature of the metabolic products varies widely, depending upon the organism, substrate, and fermentation conditions, and, therefore, no general analytical methods can be provided. However, during the fractionation of a fermentation broth, carbon recoveries were checked by carbon determinations by the wet combustion method (16).

The cell material is composed mainly of protein, lipids, carbohydrate, chitin, and sterols. In this section, only the methods for cell analyses will be described.

The cell mass, after being separated from the fermentation broth by filtration or centrifugation, is washed thoroughly with distilled water and dried under vacuum at 60°C. It is advisable sometimes to rinse the cells with a small amount of methanol or ethanol before drying.

For the determination of total nitrogen and carbohydrate, 20 mgm. of cell sample is dissolved in 1 ml. of cold 72% sulphuric acid, and rapidly diluted with distilled water to a definite volume. Aliquots are taken for the determination of cell carbohydrate with the anthrone reagent (8), with glucose as standard, and for the determination of total nitrogen by a micro-Kjeldahl method.

Cell chitin is estimated by determining residual cell nitrogen after exhaustive extraction with hot potassium hydroxide (10%), according to the procedure recommended by Norman and Peterson (9). About 30 to 50 mgm. of a cell sample are used in this determination. The chitin content is calculated with the following formula:

$$\% \text{ chitin} = \frac{\text{residual nitrogen}}{\text{weight of cell sample}} \times \frac{830}{56} \times 100.$$

The protein content of the cell is arbitrarily assumed as:

$$\% \text{ protein} = \frac{(\text{total nitrogen} - \text{chitin nitrogen})}{\text{weight of cell sample}} \times 625.$$

For lipid determination, a larger amount of sample (about 1.0 gm., depending upon the lipid content of the cell) is used. The sample is first hydrolyzed with 1*N* hydrochloric acid at a temperature between 100° and 125°C. for

about four hours. The cell residue is collected on a filter paper and washed free from acid with cold distilled water. The hydrolyzed cell mass is then dried in a vacuum oven at 60°C. The dried material is extracted with ether, and the extracted lipid is then dried over phosphorus pentoxide *in vacuo* before being weighed. The lipid is then examined for the amount and neutralization equivalent of free fatty acids, amount and saponification equivalent of neutral fat, and the amount of nonsaponifiable material. Standard methods on a reduced scale are employed. If the nonsaponifiable material constitutes only a minor fraction of the lipid, and if the lipid content of the cell is low, then the former is considered as ergosterol. The glycerol content of the lipid may be calculated from the neutral fat content or it may be determined directly by the method of Lambert and Neish (7). Where a larger sample of lipid is required for a detailed analysis, it may be isolated from cells cultivated in larger-scale fermentations in which the organism is grown under conditions similar to those attained in the experimental fermentation.

Oxidation–Reduction Status of a Substance

In this paper, the oxidation–reduction status of a compound or substance is designated in the same manner as described by Johnson *et al.* (5). The oxidation–reduction status (i.e. unit O/R value) of a compound is considered zero when it contains equivalent amounts of hydrogen and oxygen. When one mole of hydrogen is present in excess of oxygen, the substance is considered to have an O/R value of -1; and conversely, when $\frac{1}{2}$ mole of oxygen is present in excess of hydrogen, the substance is considered to have an O/R value of $+1$. For example, the unit O/R values (per mole) of glucose ($C_6H_{12}O_6$), citric acid ($C_6H_8O_7$), and stearic acid ($C_{18}H_{36}O_2$) are 0, $+3$, and -16 respectively. One nitrogen atom is equivalent to 1.5 atoms of oxygen and, therefore, the unit O/R values of ammonium hydroxide (NH_4OH) and urea ($(NH_2)_2CO$) are zero and $+2$ respectively. Table I lists the unit O/R values of a number of cell materials.

TABLE I

OXIDATION–REDUCTION VALUES OF SOME CELL MATERIALS

Substance	Unit O/R value
Carbohydrate	0.0
Cell protein	−1.78 per atom nitrogen
Chitin	−6.0 per mole
Fatty acid	$-\left(\frac{\text{N.E.}^* - 32}{14} - 2\right)$ per equivalent**
Ergosterol	−21 per mole
Glycerol	−1 per mole

N.E. = Neutralization equivalent.

***This equation is established on the assumption that the fatty acids are saturated. If lipid is considered a major product, then corrections for unsaturation should be made.*

Experimental and Results

It is obvious that, with a limited effort in the analysis of cell material, oxidation–reduction balances can be made with reasonable accuracy only for those fermentations in which (*a*) the substrate is composed of compounds of known identity; (*b*) only small amounts of proteinaceous material are produced; (*c*) the cell material is composed mainly of carbohydrate, lipid, protein, and chitin; and (*d*) the cell carbohydrate consists chiefly of glucose units. These specifications would appear to confine the study to a very limited number of fermentations. However, an experiment with a group of fungi (of different classes and genera) showed that for each mold, more than 80% of the cell material was accounted for by carbohydrate, protein, lipid, and chitin (see Table II). Also, the fungal carbohydrates were found to consist essentially

TABLE II

CELL COMPOSITION OF VARIOUS ORGANISMS

Culture	%				
	Carbohydrate**	Chitin	Protein	Lipid	Total
P. chrysogenum Q176	60.0	10.6	16.4	1.4	88.4
A. niger NRRL 337	62.0	11.9	10.8	2.2	86.9
Alternaria tenuis PRL 369	38.0	13.0	21.0	12.6	84.6
Streptomyces sp. PRL 376	24.0	7.0	46.2	8.1	86.3
Gliocladium roseum PRL 86	34.4	8.4	21.2	22.4	86.4
Ustilago zeae PRL 119*	66.2	2.9	15.6	10.0	94.7

Fermentation medium: Czapek Dox medium with 5% glucose.

**Urea is used instead of sodium nitrate as nitrogen source.*

***Calculated as glucose.*

of glucose, regardless of the organism or the type of carbon source employed (see Table III). (The latter finding made possible the use of the convenient anthrone reagent for the determination of cell carbohydrate.) Thus, conditions (*c*) and (*d*) seem to impose very little limitation. Nevertheless, one should keep in mind that these conclusions are derived from a limited number of experiments, and their significance should be re-examined when necessary.

Examples of Carbon and O/R Balances for Aerobic Fermentations

For the purpose of demonstration, carbon and O/R balances were made for (*a*) citric acid production by *A. niger*, and (*b*) ustilagic acid production by *Ustilago zeae*.

In the citric acid fermentation, a standard strain, *A. niger* 72-4, was used, and the fermentation conditions were essentially the same as those described by Shu and Johnson (13). To simplify calculations, ammonium nitrate was substituted by an equivalent amount of ammonium chloride. Citric acid and

TABLE III

EFFECT OF CARBOHYDRATE SOURCE ON THE CELL CARBOHYDRATE COMPOSITION

Culture	Glucose			Galactose			Xylose		
	% Cell carbohydrate calc'd as glucose		Sugar found in cell hydrolyzate***	% Cell carbohydrate calc'd as glucose		Sugar found in cell hydrolyzate***	% Cell carbohydrate calc'd as glucose		Sugar found in cell hydrolyzate***
	By Somogyi's method*	By anthrone reagent**		By Somogyi's method*	By anthrone reagent**		By Somogyi's method*	By anthrone reagent**	
P. chrysogenum Q176	62.0	60.0	Glucose	55.0	54.0	Glucose	56.0	60.0	Glucose
A. niger NRRL 337	64.0	62.0	"	62.0	60.5	"	64.4	62.0	"
Alternaria tenuis PRL 369	36.7	38.0	"	36.2	31.0	"	38.9	41.8	"
Streptomyces sp. PRL 376	25.2	24.0	"	No growth		—	20.4	19.0	"
Gliocladium roseum PRL 86	35.6	34.4	"	37.8	36.0	Glucose	27.8	28.8	"
Mortierella sp. PRL 26	19.3	18.7	"	No growth		—	No growth		—

Fermentation medium: Czapek Dox medium.

**Reducing sugar determined by Somogyi's method (14) on cell hydrolyzate, using glucose as standard.*

***Total carbohydrate determined with anthrone reagent (8) on cell using glucose as standard.*

****Identified by paper chromatography (4, 12) of cell hydrolyzate.*

sugar determinations were made according to the methods described by Perlman *et al*, (10) and Somogyi (14), respectively. The extracellular polysaccharide was estimated by the difference between the total carbohydrate content of the broth, as determined with anthrone reagent (8), and the residual sugar in the broth as determined by Somogyi's method (14). For the purpose of identification, the polysaccharide was precipitated from the broth with 80% ethanol. It was then freed from residual sugar by repeated precipitation in water, and hydrolyzed by the method described by Dorée (2) for cellulose hydrolysis. The hydrolyzate, after being neutralized with barium carbonate, was concentrated and analyzed for carbohydrate by paper chromatography (4, 12). Only glucose was found.

For the ustilagic acid fermentation, *Ustilago zeae* PRL 119 was employed. The medium of Thorn and Haskins (15) was used except that corn steep liquor was omitted. Ustilagic acid was determined by the method described in the same paper. Lipid determination was made on 75 ml. of broth and its composition was analyzed in the same manner as for cell lipids.

TABLE IV

CARBON AND OXIDATION–REDUCTION BALANCES ON USTILAGIC ACID FERMENTATION BY *Ustilago zeae* PRL 119

	mM.	mM. carbon	Unit O/R value per mole	Over-all O/R value
Substrate utilized				
Glucose	10.7	64.2	0.0	0.0
Urea	0.15	0.15	2.0	0.30
Oxygen	18.73	0.0	2.0	37.46
Total	——	64.35	——	37.76
Products formed				
Carbon dioxide	26.65	26.65	2.0	53.30
Ustilagic acid	0.816	30.20	−15.0	−12.24
Lipid (31.8 mgm.)				
fatty acid as stearic	0.058	1.04	−16.0	−0.93
fat as tristearin	0.016	0.91	−49.0	−0.78
nonsaponification matter as ergosterol	0.0026	0.08	−21.0	−0.06
Cell				
Cell carbohydrate as glucose	0.589	3.53	0.0	0.0
Cell chitin	0.0056	0.18	−6.0	−0.03
Cell protein (24.9 mgm.)	0.1425 mM. N_2	1.11	−1.78*	−0.51
Cell lipid (15.9 mgm.)				
fatty acid as stearic	0.029	0.52	−16.0	−0.46
fat as tristearin	0.008	0.45	−49.0	−0.39
nonsaponifiable matter as ergosterol	0.0013	0.037	−21.0	−0.03
Total	——	64.71	——	37.77

$$\textit{Carbon recovery} = \frac{64.71}{64.35} = 100.6\%.$$

$$\textit{O/R balance} = \frac{37.77}{37.76} = 1.00.$$

**Per atom nitrogen.*

The data and calculations in Tables IV and V show that carbon and O/R balances were excellent for both fermentations. No significant amounts of substances other than those listed in the tables were possibly present in the culture broths.

TABLE V

CARBON AND OXIDATION–REDUCTION BALANCES ON CITRIC ACID PRODUCTION BY *A. niger* 72-4

	mM.	mM. carbon	Unit O/R value per mole	Over-all O/R value
Substrate utilized				
Glucose	38.0	228.0	0.0	0.0
Ammonium chloride	1.96	——	0.0	0.0
Oxygen	109.3	——	2.0	218.6
Total	——	228.0	——	218.6
Products formed				
Carbon dioxide	88.20	88.20	2.0	176.4
Citric acid	16.13	96.78	3.0	48.4
Extracellular polysaccharides as glucose	2.51	15.06	0.0	0.0
Cell				
Cell carbohydrate as glucose	2.93	17.58	0.0	0.0
Cell chitin	0.114	3.65	−6.0	−0.68
Cell protein (134 mgm.)	0.765 mM. N_2	5.92	−1.78*	−2.72
Cell lipids	Trace	——	——	——
Total	——	227.2	——	221.4

$$\textit{Carbon recovery} = \frac{227.2}{228.0} = 99.7\%.$$

$$\textit{O/R balance} = \frac{221.4}{218.6} = 1.01.$$

**Per atom nitrogen.*

Estimation of Fermentation Efficiency and Respiratory Loss

For the citric acid fermentation, the respiratory quotient (total carbon dioxide produced/total oxygen uptake) is less than unity, while that of the ustilagic acid fermentation is greater than one. This is to be expected because of the oxidation reduction balance. In aerobic fermentations, the oxygen acceptors are limited to substrate carbon and substrate hydrogen but the supply of hydrogen acceptors is unlimited. For the respiratory consumption of glucose (or any other substrate of zero O/R value), the only oxygen acceptor is the substrate carbon and, therefore, the respiratory quotient equals unity. In this paper, we will use the term "respiration" to mean the conversion of substrate to the products water and carbon dioxide only. In a fermentation where carbon dioxide is the only oxidized product while the other products are highly reduced, a part of the substrate must be oxidized to carbon dioxide in order to supply hydrogen for the formation of the reduced products. Therefore, a certain amount of carbon dioxide must be formed in the fermentation process in addition to the respiratory carbon dioxide, and thus the respiratory

quotient must be greater than one. On the other hand, in a fermentation where the products other than carbon dioxide are mainly oxidized, the total oxygen uptake should be more than that for respiration alone, and hence the respiratory quotient must be less than one. In general, under ideal conditions, the carbon yield in an oxidative process should be 100%, and in a reductive process, in which an equivalent amount of oxidized carbon compounds must be formed to accompany the formation of the main product, the carbon yield must be less than 100%.

With this principle in mind, it is possible to calculate the maximum available yield of a fermentation product, which may be defined as the carbon yield of that product obtainable in an ideal fermentation in which all the substrate utilized is completely converted into (*a*) the product under investigation and (*b*) the products which must be produced in accompanying the formation of the desired product. These include cell materials, and also the products formed to fulfill the conditions for the O/R balance. When the amounts of cells formed and substrate utilized are known, the maximum available yield of a desired product and the efficiency of a fermentation may be calculated. Furthermore, the respiratory loss, i.e. the substrate carbon consumed in fungal respiration, can also be estimated.

For substrates and products of varied O/R status, the fermentation efficiencies and respiratory losses can be calculated according to the following formulae:

For $(\alpha_c - S\beta_s) \geqq 0$, $\beta_p \geqq 0$;

$$E = \frac{PaC_p}{SC_s - M_c} \tag{1}$$

For $(\alpha_c - S\beta_s) = 0$, $\beta_p < 0$;

$$E = \frac{Pa\left(C_p - \frac{\beta_p}{\beta}\right)}{SC_s - M_c} \tag{2}$$

For $(\alpha_c - S\beta_s) \neq 0$, $\beta_p < 0$;

$$E = \frac{Pa\left(C_p - \frac{\beta_p}{\beta}\right)}{SC_s - M_c + \frac{(\alpha_c - S\beta_s)}{\beta}} \tag{3}$$

For $(\alpha_c - S\beta_s) < 0$, $\beta_p > 0$, $\alpha_c < S\beta_s < 0$;
and $(\alpha_c - S\beta_s) < 0$, $\beta_p = 0$;

$$E = \frac{PaC_p}{SC_s - M_c + \frac{(\alpha_c - S\beta_s)}{\beta}} \tag{4}$$

For $(\alpha_c - S\beta_s) < 0$, $\beta_p > \beta_s \geqq 0$;

$$E = \frac{PaC_p}{SC_s - M_c + \frac{\alpha_c}{\beta}} \tag{5}$$

For $(\alpha_c - S\beta_s) < 0$, $\beta_s > \beta_p > 0$;

$$E = \frac{P_a\left(C_p - \frac{\beta_p}{\beta}\right)}{\left(SC_s - M_c + \frac{\alpha_c}{\beta}\right)\left(1 - \frac{\beta_s}{C_s\beta}\right)} \tag{6}$$

And for $\Sigma P_i\beta_i > 0$,

$$R = \frac{O\beta - \Sigma P_i\beta_i}{SC_s\beta} \tag{7}$$

For $\Sigma P_i\beta_i > 0$,

$$R = \frac{C\beta + \Sigma P_i\beta_i}{SC_s\beta} \tag{8}$$

Where $E = fermentation\ efficiency = \frac{actual\ yield}{maximum\ available\ yield}$ *for a given amount of cell formed and substrate utilized*

$M_c = mM.$ *of cell carbon formed during the fermentation*
$\alpha_c =$ *over-all* O/R *value of the cell*
$C = mM.$ *of carbon dioxide formed*
$\beta = 2 =$ *unit* O/R *value of carbon dioxide*
$S = mM.$ *of substrate fermented*
$\beta_s =$ *unit* O/R *value of substrate*
$C_s = mM.$ *carbon per* $mM.$ *substrate*
$P_a =$ *actual yield in* $mM.$ *of the product under investigation*
$\beta_p =$ *unit* O/R *value of the product under investigation*
$C_p = mM.$ *carbon per* $mM.$ *product under investigation*
$O = mM.$ *oxygen utilized*
$P_i = mM.$ *of any product, other than carbon dioxide, actually produced, where* $i = 1, 2, 3 \ldots n$
$\beta_i =$ *unit* O/R *value of product* P_i
$R =$ *fraction of substrate carbon consumed in respiration.*

Using the data given in Tables IV and V, the calculations of fermentation efficiencies and respiratory losses of the citric acid and ustilagic acid fermentations are illustrated below.

In the ustilagic acid fermentation, ustilagic acid, the product under investigation, is highly reduced; the unit O/R value of the main substrate, glucose, is zero; and the over-all O/R value of the cell is less than zero, i.e. $\beta_p < 0$, $(\alpha_c - S\beta_s) < 0$. Thus, Equation 3 may be applied for the calculation of the fermentation efficiency. Thus,

$$E = \frac{P_a\left(C_p - \frac{\beta_p}{\beta}\right)}{SC_s - M_c + \frac{(\alpha_c - S\beta_s)}{\beta}}$$

$$= \frac{0.816\left(37 + \frac{15}{2}\right)}{64.2 - 5.83 - \frac{1.42}{2}}$$

$$= 0.63.$$

Since no oxidized product other than carbon dioxide is produced in the fermentation, i.e. $\Sigma\ P_i\beta_i < 0$, the respiratory loss may be calculated with Equation 8.

$$R = \frac{C\beta + \Sigma\ P_i\beta_i}{SC_s\beta}$$

$$= \frac{53.3 - 15.43}{64.2 \times 2}$$

$$= 0.295.$$

On the contrary, in the citric acid fermentation, $\beta_p > \beta_s = 0$ and $(\alpha_c - S\beta_s) < 0$, and Equation 5 may be used for the calculation of fermentation efficiency.

$$E = \frac{P_a C_p}{SC_s - M_c + \frac{\alpha_c}{\beta}}$$

$$= \frac{16.13 \times 6}{228.0 - 27.15 - \frac{3.4}{2}}$$

$$= 0.49.$$

Since $\Sigma\ P_i\beta_i > 0$, Equation 7 may be used for the calculation of respiratory loss. Thus,

$$R = \frac{O\beta - \Sigma P_i\beta_i}{SC_s\beta}$$

$$= \frac{218.6 - 45.0}{228.0 \times 2}$$

$$= 0.38.$$

For $(\alpha_c - S\beta_s) < 0$, $\beta_p > \beta_s \geqq 0$;

$$E = \frac{PaC_p}{SC_s - M_c + \frac{\alpha_c}{\beta}} \tag{5}$$

For $(\alpha_c - S\beta_s) < 0$, $\beta_s > \beta_p > 0$;

$$E = \frac{P_a\left(C_p - \frac{\beta_p}{\beta}\right)}{\left(SC_s - M_c + \frac{\alpha_c}{\beta}\right)\left(1 - \frac{\beta_s}{C_s\beta}\right)} \tag{6}$$

And for $\Sigma\ P_i\beta_i > 0$,

$$R = \frac{O\beta - \Sigma\ P_i\beta_i}{SC_s\beta} \tag{7}$$

For $\Sigma\ P_i\beta_i > 0$,

$$R = \frac{C\beta + \Sigma\ P_i\beta_i}{SC_s\beta} \tag{8}$$

Where E = *fermentation efficiency* = $\frac{\textit{actual yield}}{\textit{maximum available yield}}$ *for a given amount of cell formed and substrate utilized*

M_c = *mM. of cell carbon formed during the fermentation*

α_c = *over-all O/R value of the cell*

C = *mM. of carbon dioxide formed*

$\beta = 2$ = *unit O/R value of carbon dioxide*

S = *mM. of substrate fermented*

β_s = *unit O/R value of substrate*

C_s = *mM. carbon per mM. substrate*

P_a = *actual yield in mM. of the product under investigation*

β_p = *unit O/R value of the product under investigation*

C_p = *mM. carbon per mM. product under investigation*

O = *mM. oxygen utilized*

P_i = *mM. of any product, other than carbon dioxide, actually produced, where* $i = 1, 2, 3 \ldots n$

β_i = *unit O/R value of product* P_i

R = *fraction of substrate carbon consumed in respiration.*

Using the data given in Tables IV and V, the calculations of fermentation efficiencies and respiratory losses of the citric acid and ustilagic acid fermentations are illustrated below.

In the ustilagic acid fermentation, ustilagic acid, the product under investigation, is highly reduced; the unit O/R value of the main substrate, glucose, is zero; and the over-all O/R value of the cell is less than zero, i.e. $\beta_p < 0$, $(\alpha_c - S\beta_s) < 0$. Thus, Equation 3 may be applied for the calculation of the fermentation efficiency. Thus,

$$E = \frac{P_a\left(C_p - \frac{\beta_p}{\beta}\right)}{SC_s - M_c + \frac{(\alpha_c - S\beta_s)}{\beta}}$$

$$= \frac{0.816\left(37 + \frac{15}{2}\right)}{64.2 - 5.83 - \frac{1.42}{2}}$$

$$= 0.63.$$

Since no oxidized product other than carbon dioxide is produced in the fermentation, i.e. $\Sigma P_i\beta_i < 0$, the respiratory loss may be calculated with Equation 8.

$$R = \frac{C\beta + \Sigma P_i\beta_i}{SC_s\beta}$$

$$= \frac{53.3 - 15.43}{64.2 \times 2}$$

$$= 0.295.$$

On the contrary, in the citric acid fermentation, $\beta_p > \beta_s = 0$ and $(\alpha_c - S\beta_s) < 0$, and Equation 5 may be used for the calculation of fermentation efficiency.

$$E = \frac{P_a C_p}{SC_s - M_c + \frac{\alpha_c}{\beta}}$$

$$= \frac{16.13 \times 6}{228.0 - 27.15 - \frac{3.4}{2}}$$

$$= 0.49.$$

Since $\Sigma P_i\beta_i > 0$, Equation 7 may be used for the calculation of respiratory loss. Thus,

$$R = \frac{O\beta - \Sigma P_i\beta_i}{SC_s\beta}$$

$$= \frac{218.6 - 45.0}{228.0 \times 2}$$

$$= 0.38.$$

The calculations of the fermentation efficiencies as shown above are based chiefly on the material balances. No consideration is made for the free energy change involved in the fermentations. If the fermentation process is highly endergonic, the free energy requirement of the process must be obtained from respiration, and a considerable amount of respiration must occur. The fermentation efficiency calculated by the above formulae will then deviate from the true value. Nevertheless, these values will still be closer to the true values than those obtained by other existing methods.

The extent of the free energy input of a fermentation may be observed from the magnitude of the respiratory loss. The synthesis of reduced products is likely an endergonic process, and considerable respiration may be expected to accompany the process. Thus, under optimum fermentation conditions, it may be expected that the respiratory loss in citric acid production is lower than that in yeast cell production, and the latter, in turn, is lower than that in fat production. (Among these, citric acid has the highest O/R value while fat has the lowest.) Accordingly, the carbon yield is expected to be the highest in citric acid production and the lowest in fat production. This agrees with the fact that a citric acid yield as high as 90% has been obtained (17) while, in yeast and fat production, maximum yields of no more than 65% and 18% respectively have been reported in the literature (1, 3). (These values are roughly equivalent to 97%, 90%, and 70% fermentation efficiencies, respectively.)

From the above discussion, it can be seen that the results from carbon and oxidation–reduction balances, coupled with the calculations of fermentation efficiency and respiratory loss, are helpful in visualizing the general picture of a fermentation. Such results could be of use, also, in estimating possible further improvements in a given fermentation.

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CLOSTERIUM IN CENTRAL CANADA[1]

By Elwyn O. Hughes[2]

Abstract

Fifty-seven species and varieties of *Closterium* are reported from Manitoba and Ontario. Previous reports from central, eastern, and arctic Canada are cited. Each species is illustrated and measurements are given from the author's material. The text and catalogue of species include notes on taxonomy of the genus. Two new species, four new varieties, and one new form are described.

Introduction

Although the genus *Closterium* is a common constituent of the fresh-water algal flora, reports of its presence in central Canada have been infrequent. Twenty-one species and varieties have been reported from Ontario by Bigelow (2, 3, 4), Lowe (28), Mackenzie (30), Smith (47), and Taft (51). In addition Lowe (28) recorded three species specifically from Manitoba. By way of contrast, over seventy species and varieties are known to occur in Quebec (6, 7, 18, 19, 20, 21, 23, 24, 29, 32); over fifty in the Maritime Provinces (17); and over thirty in Newfoundland (52). About twenty-five species and varieties have been reported from various points in the Canadian Arctic (11, 27, 57).

The present study includes a catalogue of 57 species and varieties of *Closterium* from central Canada. All but two of these entities occur in the author's collections. Thirty-three are new records for the province of Manitoba and 25 new to Ontario. Thirteen of the plants apparently have not been reported previously from Canada and seven new entities are described.

All collections were preserved in Transeau's solution although much of the material collected at Churchill, Man., was examined in the living condition before preservation. Camera lucida drawings were made of all species seen. Representative drawings of each entity are appended (Figs. 1–60). Since these records were made using different microscopes at four different laboratories, there is some variation in magnification.

Taxonomic Notes

Identification of the species of *Closterium* is based upon the following characteristics:

(i) The curvature of the cell. In general, it is sufficient to know whether the cell is *strongly* or *weakly* curved. Curvature may, however, be measured in degrees of the arc formed by the dorsal wall, using a closteriocurvimeter (14). A more useful measurement would include the radius of curvature.

(ii) The size of the cell. Under this heading is included the length (a direct line between the apices), the width (at point of maximum breadth),

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and the width of the apex. The latter measurement should be taken at a point 5 μ from the apex except in the case of truncate or capitate varieties.

(iii) The shape of the cell, i.e. the length/width ratio, amount of median tumescence, degree of attenuation, shape of the apices, etc.

(iv) The ornamentation of the cell wall, e.g. plain, punctate, striate, costate, etc. The ornamentation of apparently plain walls is often visible only under oil immersion with optimum illumination, e.g. the striae of *C. praelongum* var. *brevius*, *C. macilentum* var. *substriatum*, and some specimens of *C. intermedium*. The number of striae or costae per 10μ is also determined more accurately under oil.

(v) The type of cell division, i.e. presence or absence of median girdles. This is not a constant characteristic but usually holds for the majority of members of a given species. Sutures due to ordinary cell division should not be confused with girdles.

(vi) The structure of the chromatophore, i.e. the number of longitudinal ridges, and the number and arrangement of pyrenoids.

(vii) The size and ornamentation of the zygospore. No zygospores were observed in the author's collections.

(viii) The number of crystals in the terminal vacuoles. The author feels that this characteristic is of no systematic value.

The author is not aware of any species of *Closterium* in which all of the above characteristics are absolutely constant. Since the description of a species is, in itself, a cumulative expression of a number of variables, it is not surprising to find frequent references in the literature to intergrading forms.

In the matter of determining the category to which a new variant should be assigned, the literature affords little help. Some authors create new varieties on the basis of size differences (e.g. var. *minus* or var. *majus*), others on the basis of difference in ratio of length to width (e.g. var. *angustius* or var. *tenuius*). Sometimes variation in pyrenoid arrangement is considered to be varietal (e.g. *C. moniliferum* var. *submoniliferum*), at other times specific (e.g. *C. lunula* vs. *C. pseudolunula*). Some authors have created new species on the basis of zygospore differences only (e.g. *C. dianae* vs. *C. calosporum*). In such cases, it is impossible to identify the plant on the basis of its vegetative characteristics. Others consider such differences to be merely varietal or even formal (e.g. *C. tumidum* var. *nylandicum* fa. *macrosporum*). Many authors consider variation in number of striae per 10 μ warrants specific differentiation (e.g. *C. intermedium* vs. *C. striolatum*). Others include striate and smooth-walled plants in different varieties of the same species (e.g. *C. cynthia* and var. *jenneri*), or even within one variety (e.g. *C. ralfsii* (24)).

Despite these inconsistencies, it is quite possible that the majority of *Closterium* species, as presently understood, are valid taxonomic entities. But it is also quite certain, in the absence of cultural studies, that we have no convincing evidence of this validity. We do have a mass of evidence of

variability within the genus. A system of nomenclature has been set up to record these variations and with it we must be content for the time being.

Source of Collections

Collections used in this study were made by the author as follows:

Southern Ontario (June 13–18, 1939)

Ponds: Brantford—1, 2

Maitland River: Cranbrook—3, 5, 6

Brooks: Gorrie—7, 8, 10

Northern Ontario

Pools in *Sphagnum* bogs

Aug. 30, 1938: Michipicoten River—MR-1.

June 7–12, 1940: Powassan—103, 124; Trout Lake—234; twenty miles south of Timagami—230, 231; Englehart—244; Swastika—236; Holland—249, 253; Crown Timber Area south of Cochrane—252, 263; two miles northeast of Cochrane—130, 131, 153; two miles north of Clute—132, 150, 151; Blount—141.

Brooks (June 7–12, 1940)

One mile north of Holland—240, 241; Edgmere—106; Blount near Abitibi River—139.

Lake shore

Five miles north of Timagami—239.

Gravel pit

Cochrane—122.

Northern Manitoba (June 7–July 27, 1949)

Pools in heath tundra (along coast between Churchill and Fort Churchill): C-104, C-106, C-107, C-108, C-109, C-127, C-128, C-207, C-246, C-260, C-261, C-262, C-266.

Pools in sedge meadow: Churchill, southeast of Lake Isabelle,—C-188, C-189, C-281, C-293; near the south and south-southeast borders of the airstrip—C-190, C-223, C-225, C-256.

Spruce forest area—Pools near north end of Farnworth Lake (about 5 miles south of Churchill): C-181, C-183.

At points along Hudson's Bay Railroad (Churchill being at Mile 509.8): Mile 505.1—C-177; Mile 500.5—C-253; Mile 498.6—C-156; Mile 495—C-248; Mile 496 -C-204; Mile 483—C-201, C-202, C-203.

Northwest Territories (July 21, 1949). Collected by Mr. Hedley James.

Ennadai Lake: J-7.

The vegetation of the Churchill area may be roughly divided into three regions, heath tundra, sedge meadow, and spruce forest. A general description with a vegetational map is given by Shelford and Twomey (46). The climax heath tundra proved to be almost sterile in so far as species of *Closterium* were concerned. Of the half dozen species found in this area nearly all were

confined to pools in the transition zone between heath tundra and the coastal cliffs. Only two collections in this area contained as many as three species. In those pools where pH was determined, the range was from pH 5 to pH 6.

The sedge meadow pools (pH 5.5 to 7.5) were not quite as poor in *Closterium* species, one collection containing as many as five. The maximum pH recorded in this area may be the result of disturbance since it was taken near the edge of a gravel road. The usual pH range was 5.5 to 6.5.

The richest collecting area for *Closterium* proved to be in the spruce forest from 5 to 23 miles south of Churchill. Several collections from this region contained from 5 to 12 species. At least two-thirds of the Churchill species of *Closterium* were found in pools (pH 5 to 6.5) along the railroad in the spruce forest.

The Northern Ontario collections were taken from points along the north–south extent of Highway No. 11. The presence in bogs of *C. moniliferum* and some other species usually confined to more alkaline waters may have been due to disturbance, since some of the collections were made near the edge of the road.

The species of *Closterium* collected in southwestern Ontario were characteristic of alkaline waters.

Annotated List of Species

The nomenclature used in this study (with some exceptions noted in the species list) is that adopted by Krieger (25). Changes have been made in some cases where the name applied by Krieger seems inconsistent with the International Rules (8). The type variety (var. *typicum* or var. *genuinum* of some authors) has been indicated by the repetition of the specific epithet as a varietal epithet, an innovation authorized by the 1950 International Congress (42).

Identifications are based chiefly on the descriptions given by Krieger (25), Irénée-Marie (18), and W. and G. S. West (55). Other literature found useful in determining the extent of variation permissible in a given taxon is cited where pertinent.

Dimensions given for each variety (L. = length, W. = width, ap. W. = width of the apex) are the extremes found in those specimens measured by the author. Distributional data are given for each variety in the author's collections and for previous records from Manitoba and Ontario. *An asterisk is used to indicate first reports from these provinces.* Additional literature citations are given for reports of the species in the remainder of eastern Canada and the Arctic.

1. *Closterium acerosum* (Schrank) Ehr. var. *acerosum* (Figs. 1*a*, 1*b*) W. 30–58 μ; ap. W. 5–7 μ; L. 350–650 μ, (L. = 8–14 W.).

   Ontario: Blount—139, 141; Brantford—2 (plentiful); Cochrane—122; Englehart—244 (plentiful); Holland—240; Shoal Lake, Lake of the Woods (28).

Manitoba: Churchill—C-223, C-256; Lake Winnipeg, Red River, Killarney (28).

Quebec: (6, 18, 19, 21, 29). Maritimes: (17). Newfoundland: (52).

In the Englehart and Brantford collections, there are 8 to 12 longitudinal ridges per chromatophore and each chromatophore contains 9 to 29 pyrenoids in an axial row. The striae are difficult to see even with oil immersion. Some specimens appear to be smooth-walled.

Lefevre (26) has demonstrated considerable polymorphism in cultural studies of this species. That similar variation occurs in nature is attested by the variation in illustrations given by Ralfs, Krieger, and the Wests. Figs. 1*a* and 1*b* indicate extremes in L./W. ratio found in the Ontario collections. Fig. 1*b* approaches the var. *elongatum*.

2. *C. acerosum* var. *elongatum* Bréb. (Figs. 2*a*, 2*b*) W. 35–38 μ; ap. W. 6–7 μ; L. 720–775 μ, (L. = 20–22 W.).

*Ontario: Englehart—244.

Quebec: (18, 24). Newfoundland: (52).

Chromatophore with 8–10 ridges and 17–30 axial pyrenoids. Wall finely striate, the apparently punctate striae merging with irregularly scattered punctae at the apices. Except for the high L./W. ratio, Fig. 2*a* is typical of var. *acerosum* as well as var. *elongatum*.

3. *C. acerosum* var. *tumidum* Krieger fa. (Fig. 7) W. 48 μ; ap. W. 6–7 μ; L. 310–375 μ, (L. = 6–7 W.).

*Manitoba: Churchill—C-204. Only two specimens seen. Wall plain; seven-eight axial pyrenoids per chromatophore; stouter than described by Krieger (25). Apparently the name var. *tumidum* has been created by Krieger. Although the latter author cites "Borge" as the author epithet, Nordstedt (34, 35) does not include this variety under those named by Borge.

4. *C. acutum* Bréb. var. *acutum* (Fig. 8) W. 4.5–5 μ; ap. W. 1.5–2.5 μ; L. 110–140 μ.

*Manitoba: Churchill—C-266.

Quebec: (18, 21, 24). Maritimes: (17).

5. *C. angustatum* Kuetz. var. *angustatum* (Fig. 42) W. 22–23 μ; ap. W. *ca.* 13 μ; L. 310–448 μ, (L. = 14–20 W.).

Ontario: Timagami—239; Muskoka (47).

Quebec: (18, 19, 21, 23, 24). Maritimes: (17). Newfoundland: (52).

FIGS. 1*a*, 1*b*. *Closterium acerosum* var. *acerosum*. FIGS. 2*a*, 2*b*. *C. acerosum* var. *elongatum*; striae *ca.* 15 in 10 μ. FIG. 3. *C. lunula* var. *lunula*. FIG. 4. *C. cornu* var. *cornu*. FIG. 5. *C. praelongum* var. *brevius*; striae *ca.* 13 in10 μ. FIG. 6. *C. pritchardianum* var. *pritchardianum*; striae *ca.* 15 in 10 μ. FIG. 7. *C. acerosum* var. *tumidum*. FIG. 8. *C. acutum* var. *acutum*. FIG. 9. *C. baillyanum* var. *baillyanum*.

Scale: *A*. Figs. 1*a*, 1*b*, 4, 5, 6, 9. *B*. Figs. 2*a*, 3. *C*. Figs. 7, 8.

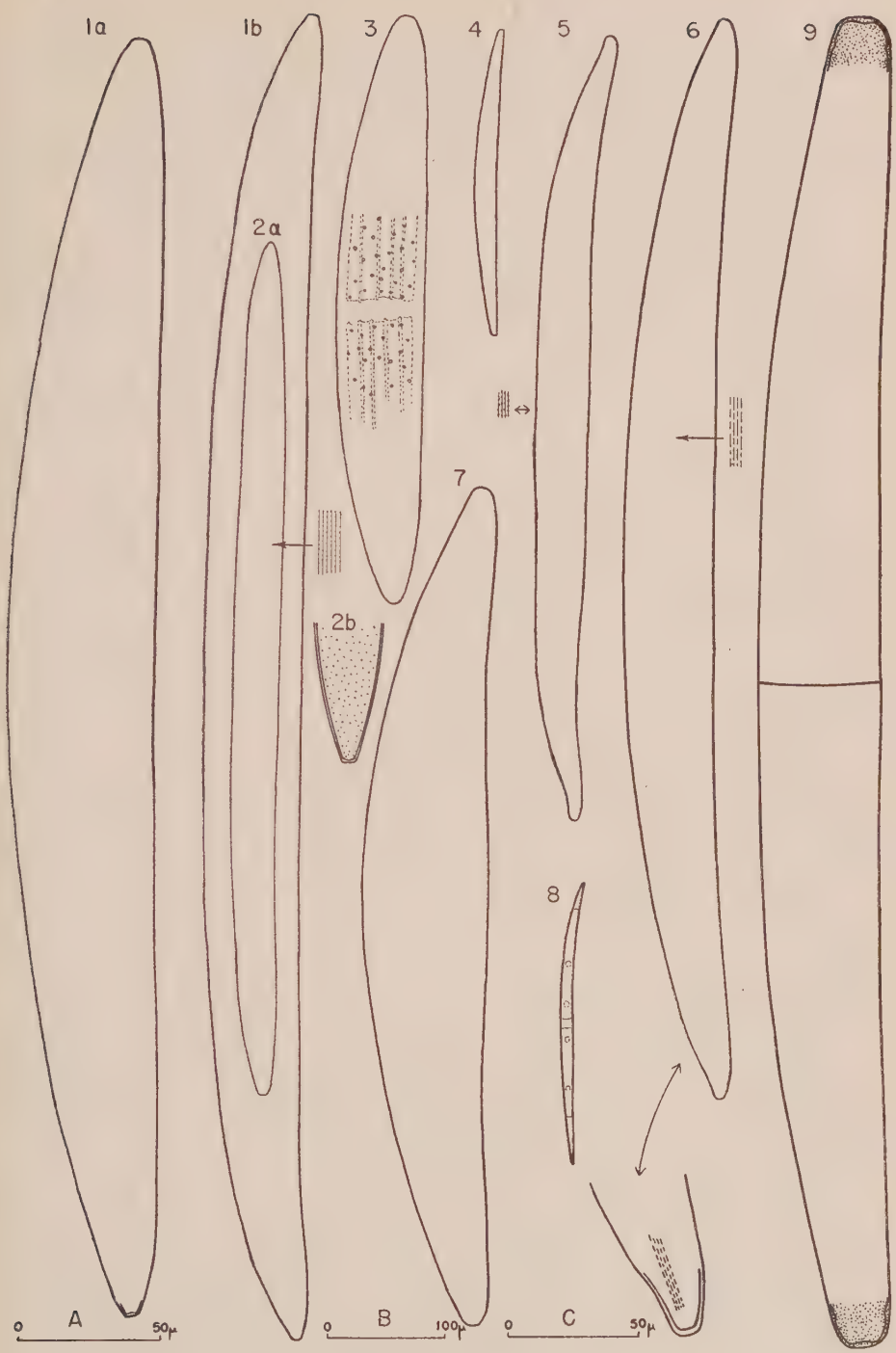

FIG. 1-9.

United States: Rhode Island, New Jersey (41); Massachusetts, Minnesota, California (59); Michigan (33, 37, 38, 53); Maine (56); Montana, Idaho, Oregon (40).

Asia: Singapore (1); Japan (15).

Synonymy:

Ophiocytium cuspidatum (Bailey) Rabenhorst 1868 (59).
Reinschiella ? cuspidata de Toni 1889 (36).
Tetraedron cuspidatum (Bailey) Wille 1897 (36).
Spinoclosterium curvatum Bernard 1909 (1).
Closterioides spinosus Prescott 1937 (37).
Spinoclosterium curvatum Bernard var. *spinosum* Prescott 1939 (38).
Spinoclosterium cuspidatum (Bailey) Hirano 1949 (15).

The taxonomy of this organism has long been confused. Several authors have referrred it to the Chlorococcales. Krieger (25) felt that it was probably a cyst of one of the Peridineae. Prescott (37, 38) appears to have been the only author fortunate enough to have examined plants in the living condition and to have found specimens undergoing division. It is now generally accepted that the plant is a desmid (15, 22, 24, 49); there is, however, some disagreement as to whether it differs enough from the other species of *Closterium* to warrant the establishment of a separate genus. The present author prefers to follow Irénée-Marie (22, 24) and Ralfs (41) in retaining the plant in the genus *Closterium*.

The rather abrupt contraction of the apices to the base of the spines in the figures of Whelden (56) and Prescott (37) produces a distinct dorsal hump. Sometimes a similar hump is produced ventrally. The figures of Wolle (59), of Bernard (1), and of Hirano (15) show no such humping. In Prescott's Fig. 1 (37) the apices appear to be almost swollen. There is a slight suggestion of a dorsal hump at the upper apex of Bailey's figure (41). The variety *spinosum* Prescott is based on the presence of the hump. But if Ralfs intended a hump in his figure, the var. *spinosum* is, in fact, the var. *cuspidatum* and, as pointed out by Irénée-Marie (22), Bernard's material would have to be a variety or form of the type. This confusion may quite possibly be due to inaccurate drawings in the earlier works. Sometimes too, drawings change in form when transferred from one sheet of paper to another. In this connection see Pascher's (36) figure of the species "after Ralfs". Rather than add to the confusion, the author prefers to retain the original specific name which in any case is now (42) considered to include all taxa of infraspecific rank.

FIG. 23. *Closterium arcuarium*. FIG. 24. *C. cynthia* var. *jenneri*. FIG. 25. *C. tumidum* var. *nylandicum*. FIG. 26. *C. pusillum* var. *pusillum*. FIG. 27. *C. arcuarium*. FIGS. 28, 29. *C. macilentum* var. *substriatum*; striae *ca.* 17 in 10 μ. FIG. 30. *C. cynthia* var. *cynthia*. FIGS. 31, 32. *C. intermedium* var. *intermedium*. FIG. 33. *C. intermedium* var. *hibernicum*. FIG. 34. *C. planum*. FIGS. 35, 36. *C. striolatum* var. *striolatum*.

Scale: *A*, Figs. 23, 24, 26, 27, 28, 31. *B*. Figs. 25, 30. *C*. Figs. 29, 32, 33, 34, 35, 36.

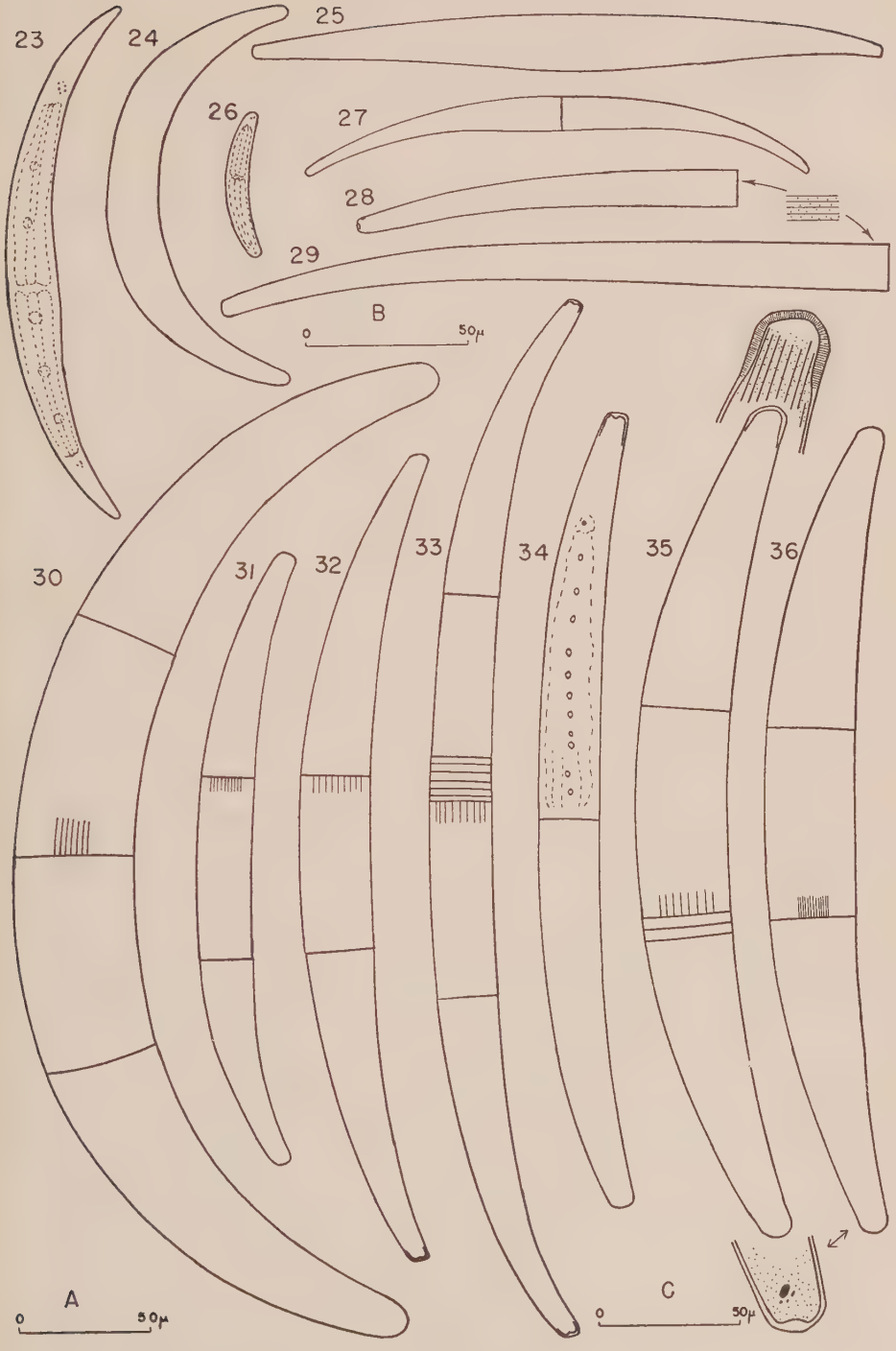

FIG. 23–36.

12. *C. cynthia* de Not. var. *cynthia* (Fig. 30) W. 18–21 μ; ap. W. 4–5 μ; L. 140–145 μ.

*Manitoba: Churchill—C-202, C-253.

Arctic: (11, 57). Quebec: (24). Maritimes: (17). Newfoundland: (52).

13. *C. cynthia* var. *jenneri* (Ralfs) Krieger (Fig. 24) W. 7–18 μ; L. 43–110 μ.

Ontario: Holland—253; Toronto (30); Mud Lake (28).

Quebec: (18, 19, 29, 32). Arctic: (27, 57). Maritimes: (17).

The dimensions cited above are from Krieger (25). The illustration is of a plant larger (19 $\mu \times 146$ μ) than previously assigned to this variety.

The reader is referred to Grönblad (12) for reasons for retaining *C. jenneri* as a specific entity rather than as a variety of *C. cynthia*. Prescott and Scott (39) refer to "fine striations" in this species (*ca.* 12 in 10 μ). Grönblad (13) refers to plain walls even with oil immersion.

14. *C. dianae* Ehr. var. *dianae* (Fig. 15) W. 18–20 μ; ap. W. *ca.* 3–5 μ; L. 175–210 μ.

*Manitoba: Churchill—C-203, C-253.

Ontario: Toronto (30, as *C. acuminatum*), Muskoka (28).

Arctic: (11, 27, 57). Quebec: (18, 19, 23, 24, 29). Maritimes: (17). Newfoundland: (52).

15. *C. dianae* var. *arcuatum* Bréb. (Fig. 56).

Ontario: Toronto (30).

Quebec: (18, 19, 21, 24). Arctic: (57).

16. *C. dianae* var. *pseudodianae* (Roy) Krieger (Fig. 20) W. 12–17 μ; ap. W. 3–4 μ; L. 185–260 μ.

*Manitoba: Churchill—C-202, C-203.

*Ontario: Holland—253.

Quebec: (18, 19, 24). Maritimes: (17). Newfoundland (52). Arctic: (11, 57).

17. *C. eboracense* Turn. (Fig. 16) W. 55 μ; ap. W. *ca.* 10 μ; L. 246 μ.

Ontario: Cochrane—122; Toronto (30, as *C. cucumis* Ehr.).

Newfoundland: (52).

18. *C. ehrenbergii* Menegh. var. *ehrenbergii* (Fig. 17) W. 60–83 μ; ap. W. *ca.* 12–14 μ;. L. 320–490 μ.

Pyrenoids scattered in the chromatophore.

*Manitoba: Churchill—C-156, C-181.

Ontario: Blount—141; Brantford—2; Cochrane—252, 263; Cranbrook—3; Timagami—230; Toronto (30); Mud Lake (28); Muskoka (47).

Quebec: (18, 19, 21, 23, 24, 29). Maritimes: (17).

19. *C. ehrenbergii* var. ***pseudopodolicum*** var. nov. (Fig. 58).

Cellulis vegetativis 84–90 μ lat., 400–425 μ long.; longitudine cellularum circa 4– ad 5– plo latitudine; apicibus (10–12 μ lat.) in conos rotundatos subito contractis. Cetero ut in var. *ehrenbergii*.

*Ontario: Brantford—1.

This variety should be compared with Gutwinski's var. *podolicum* as described by Krieger (25). Krieger includes Deflandre's (10) *C. ehrenbergii* fa. under the latter variety.

C. ehrenbergii var. *pseudopodolicum* differs from the var. *podolicum* in that the apices are suddenly contracted dorsally, ventrally, and laterally, and are not recurved. The wall thickenings present in the apices of Deflandre's specimens are not present in the var. *pseudopodolicum* and the dimensions given by Deflandre are considerably larger (W. 114–134 μ × L. 554–649 μ).

In the Ontario specimens there are 12 to 16 rather irregularly defined longitudinal ridges in each chromatophore, and the pyrenoids are numerous and scattered. Specimens are fairly frequent in the collection and no other varieties of *Closterium* are present.

20. *C. gracile* Bréb. var. *gracile* (Fig. 39) W. 4.5–8.5 μ; ap. W. 2–3 μ; L. 108–261 μ.

*Manitoba: Churchill—C-156, C-202, C-203, C-204.

Ontario: Holland—253; Timagami—239; Toronto (30).

Quebec: (18, 19, 21, 23, 24, 29). Maritimes: (17). Newfoundland: (52).

21. *C. idiosporum* W. and G. S. West var. *idiosporum* (Fig. 38) W. 10–11 μ; ap. W. 2–3 μ; L. 179–284 μ.

*Manitoba: Churchill—C-128, C-204.

Quebec: (18, 24).

In collection C-128 very few of the cells have the slightly swollen middle referred to by Krieger (25) but his Fig. 1 does not show this feature. Some of the cells are slightly recurved resembling Krieger's Fig. 2 of conjugating cells. Coll. C-204 is typical.

22. *C. intermedium* Ralfs var. *intermedium* (Figs. 31, 32) W. 21–26 μ; ap. W. 6–8 μ; L. 195–300 μ.

*Manitoba: Churchill—C-246, C-252, C-261.

Ontario: Cochrane—252, 263; Holland —253; Shoal Lake and Mud Lake (28).

Quebec: (18, 19, 21, 24, 29). Maritimes: (17).

The identification of this species is discussed under *C. striolatum*. Although specimens were plentiful in collection C-252, striae were barely visible even with the use of the oil immersion objective.

23. *C. intermedium* var. *hibernicum* W. West (Fig. 33) W. 17–25 μ; ap. W. 6–8 μ; L. 250–370 μ.

*Manitoba: Churchill—C-253, C-203.

*Ontario: Cochrane—252, 263; Timagami—239.

The variety was named on the basis of the greater L./W. ratio and the somewhat coarser striations (55). Krieger (25) states that the greater L./W. ratio is due to the interpolation of additional girdles (as in our figure). He also states that the wall is not always so coarsely striate as described by the Wests. In the Timagami collection and in Churchill collection C-203, cells with only one girdle are quite slender (L. = 14–18 W.) and the striae are quite coarse.

24. *C. juncidum* Ralfs var. *brevius* Roy (Fig. 41) W. 12 μ; ap. W. 6 μ; L. 198 μ.

*Ontario: Timagami—239.

Arctic: (11).

25. *C. kuetzingii* Bréb. var. *kuetzingii* (Fig. 46) W. 18–24 μ; ap. W. 3–4 μ; L. 375–525 μ.

*Manitoba: Churchill—C-189, C-202, C-203, C-253.

Ontario: Timagami—239; Pelee Island (51); Mud Lake (28).

Quebec: (18, 19, 21, 23, 24). Maritimes: (17). Arctic: (11, 57).

26. *C. kuetzingii* var. *laeve* (Rac.) Kütz. W. 16 μ; ap. W. 3–3.5 μ; L. 480–497 μ.

*Manitoba: Churchill—C-156. Apparently new to North America.

Differs from the var. *kuetzingii* only in the absence of longitudinal striations. Striae were not seen in our specimens even with oil immersion (× 1800).

27. *C. lanceolatum* Kütz. (Fig. 52) W. 42–73 μ; ap. W. *ca.* 6 μ; L. 285–465 μ; chromatophore with eight longitudinal ridges and 8–12 axial pyrenoids.

*Ontario: Cranbrook—5; London (collection lost).

Manitoba: Lake Winnipeg (28).

Quebec: (18, 19, 21, 24). Maritimes: (17). Arctic: (57).

28. *C. laterale* Nordst. var. ***simplicius*** var. nov. (Fig. 50).

Cellulis vegetativis 30 ad 45 μ lat., 425 ad 530 μ long.; apicibus truncatis 6 ad 8 μ lat. Pyrenoidibus in medio chromatophori 6 ad 8 in ordine positis. Chromatophoris circa 6 lamellis longitudinalibus. Membranis cellularum striatis, striis 8–14 in 10 μ. Cetero ut in var. *laterale*.

W. 30–45 μ; ap. W. 6–8 μ; L. 432–530 μ, (L. = 10–16 W.).

*Manitoba: Churchill—C-203.

FIG. 37. *Closterium ralfsii* var. *kriegeri*. FIG. 38. *C. idiosporum* var. *idiosporum*. FIG. 39. *C. gracile* var. *gracile*. FIG. 40. *C. pronum* var. *pronum*. FIG. 41. *C. juncidum* var. *brevius*; striae *ca.* 15 in 10 μ. FIG. 42. *C. angustatum* var. *angustatum*. FIG. 43. *C. tumidum* var. *tumidum*. FIGS. 44, 45. *C. tumidum* var. *nylandicum*. FIG. 46. *C. kuetzingii* var. *kuetzingii*. FIG. 47. *C. rostratum* var. *rostratum*. FIG. 48. *C. setaceum* var. *setaceum*. FIG. 49. *C. lineatum* var. *lineatum*. FIG. 50. *C. laterale* var. *simplicius*. FIG. 51. *C. striolatum* var. *subtruncatum*. FIG. 52. *C. lanceolatum* var. *lanceolatum*.

Scale: *A*. Figs. 37, 51. *B*. Fig. 40. *C*. Figs. 38–39, 41–50, 52.

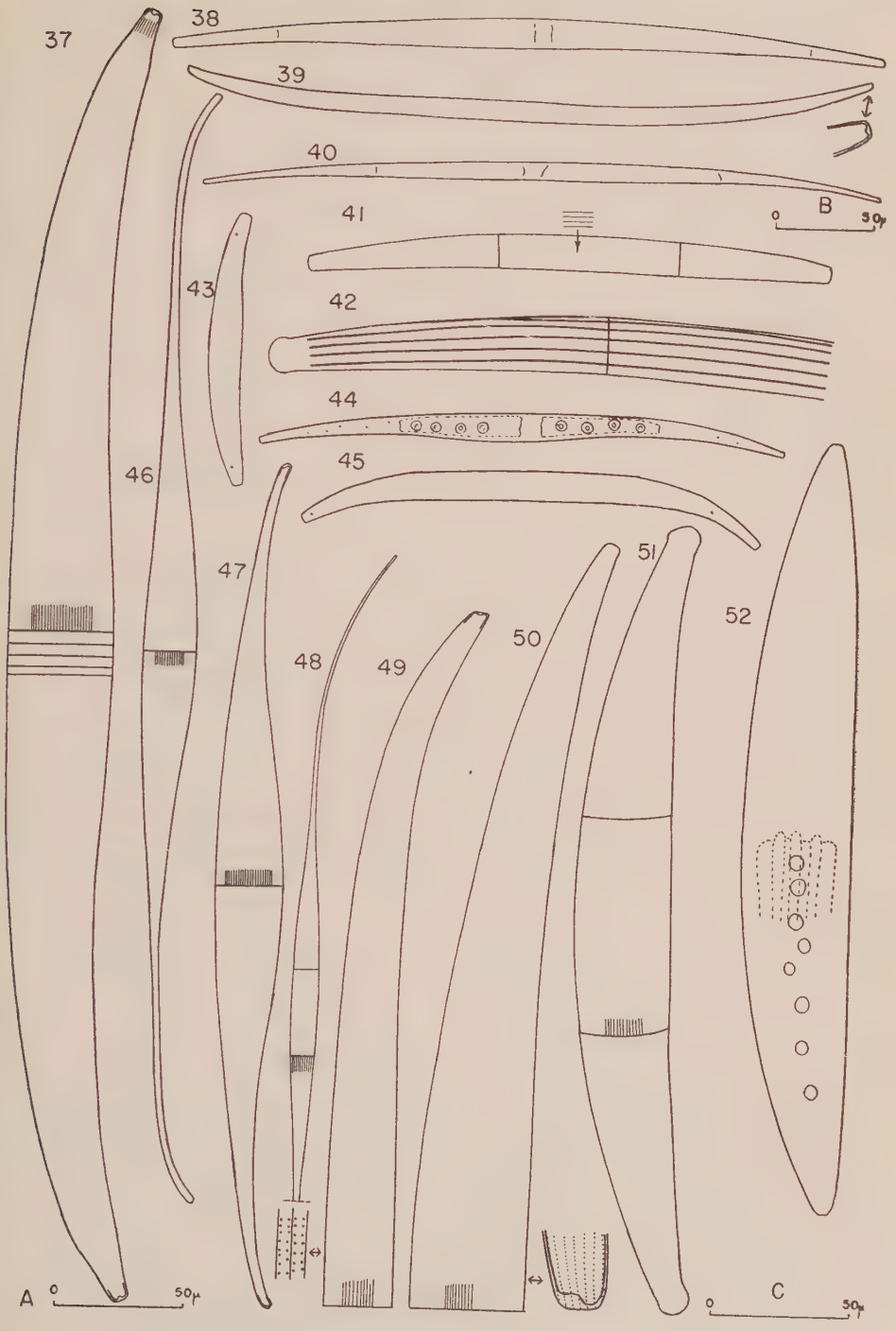

FIG. 37-52.

Grönblad (13) has published a figure (Taf. I, Fig. 11) drawn by Löfgren from Nordstedt's original material of *C. laterale*. The wall characteristics in this drawing agree very closely with those in the Churchill material even to the dorsally displaced thickening of the apical wall. *C. laterale* var. *simplicius* may be distinguished from *C. laterale* var. *laterale* by the presence in var. *simplicius* of about six longitudinal ridges on the chromatophore and by the axial row of six to eight pyrenoids in each chromatophore.

The striae of *C. laterale* var. *simplicius* are easily seen with a high dry objective (× 500). Under oil immersion (× 1100) the striae appear to be composed of parallel rows of granules (or punctae). These "granules" are so close to the limits of resolution that their exact structure is uncertain. There are about 8 to 14 striae in each 10 μ of the cell wall. The striae are continuous to the apices of the cells.

29. *C. leibleinii* Kütz. var. *leibleinii* (Figs. 22, 54) W. 35–48 μ; ap. W. 4–5 μ; L. 169–225 μ; pyrenoids 5–11 per chromatophore, crystals about 10–12 per apical vacuole.

*Ontario: Gorrie—7, 8; Cranbrook—3, 6.

Quebec: (18, 19, 21, 24). Maritimes: (17). Arctic: (11, 57).

This material agrees in all respects with Ralf's description (41) of the species. Grönblad (12) points out that Krieger (25) appears to have included in his diagnosis, forms that are indistinguishable from *C. moniliferum*. Ralfs states that the median inflation is often "nearly obsolete". Such is the case in the majority of the Gorrie specimens, although a few were seen with a very slight inflation. In the Cranbrook specimens, on the other hand, the median inflation was more prominent. The almost acute apices separate this material from anything in the same size range in the author's collections. The cells are more strongly curved than in *C. moniliferum*. Irénée-Marie's Plate 5, Fig. 7 (18) is cited for comparison.

30. *C. lineatum* Ehr. var. *lineatum* (Fig. 49) W. 16–26 μ; ap. W. 4–8 μ; L. 400–525 μ.

*Manitoba: Churchill—C-89.

Ontario: Timagami—239, Lake Nipigon (2, 4).

Quebec: (18, 19, 21, 24, 29). Maritimes: (17).

The Ontario specimens (16–18 μ × 400–500 μ) are much narrower than the Manitoba specimens (23–26 μ × 500–525 μ). The cell apices are more incurved in the Manitoba material. As many as 24 pyrenoids are present in each semicell in the Ontario specimens. The drawing is of one of the latter.

FIG. 53. *Closterium ralfsii* var. *hybridum*. FIG. 54. *C. leibleinii* var. *leibleinii*. FIG. 55. *C. costatum* var. *westii*. FIG. 56. *C. dianae* var. *arcuatum* (after Krieger (25)). FIG. 57. *C. arcuarium*. FIG. 58. *C. ehrenbergii* var. *pseudopodolicum*. FIG. 59. *C. turgidum* var. *turgidum* (after Krieger (25)); striae *ca.* 10 in 10 μ. FIG. 60. *C. cuspidatum*.

Scale: *A*. Figs. 53–55; 57, 58, 60. *B*, Fig. 56. *C*. Fig. 59.

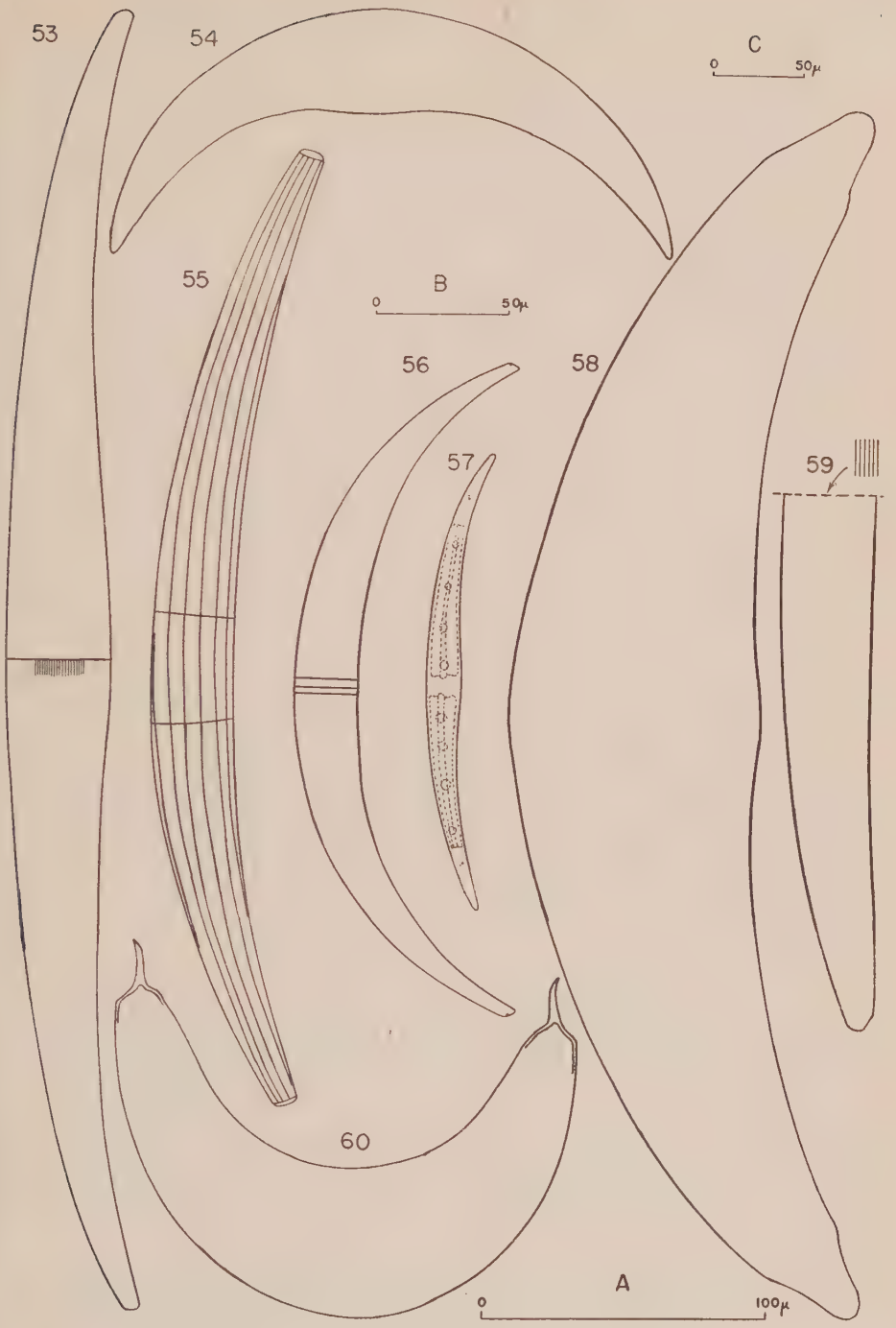

FIG. 53-60.

31. *C. lunula* (Mull.) Nitzsch var. *lunula* (Fig. 3) W. 53–100 μ; ap. W. 11–17 μ; L. 243–550 μ.

*Manitoba: Churchill—C-109.

Ontario: Michipicoten River—MR-1; Lake Nipigon (2, 4).

Quebec: (18, 19, 21, 24, 29). Maritimes: (17).

The species is frequent in the Michipicoten collection. The Churchill record is doubtful since the arrangement of the pyrenoids could not be determined with certainty.

32. *C. macilentum* Bréb. var. *macilentum* W. 11 μ; ap. W. 5 μ; L. 350 μ.

Ontario: Timagami—239; Toronto (30, as *C. brebissonii* Delp.); Lake Erie (51).

Quebec: (18, 19, 21, 24). Maritimes: (17). Arctic: (57).

Differs from var. *substriatum* (Grönbl.) Krieger (Figs. 28, 29) only in the absence of wall ornamentation.

33. *C. macilentum* var. *substriatum* (Grönbl.) Krieg. (Figs. 28, 29) W. 12–17 μ; ap. W. 5–6 μ; L. 270–475 μ.

*Manitoba: Churchill—C-203, C-204. Apparently new to North America. Only two specimens seen. Cell wall pale brown. Striae (15–20 in 10 μ) visible only under oil immersion.

34. *C. malinvernianiforme* (Grönbl.) var. ***gracilius*** var. nov. (Fig. 21).

Cellulis 32–42 μ lat., 236–310 μ long.; longitudine cellularum 7.5–9 plo latitudine; apicibus (5–7 μ lat.) truncatis et nonunquam angulo-truncatis; 6–9 pyrenoidibus in medio chromatophori in ordine positis; chromatophoris circa 8 lamellis longitudinalibus; membranis cellularum striatis, striis 7–12 (ad 20) in 10 μ; membranis apicium punctatis non striatis. Cetero ut in var. *malinvernianiforme*.

*Manitoba: Churchill—C-223 (frequent), C-225 (frequent), C–256 (rare), C-284 (rare).

*Ontario: Cochrane—122 (rare).

This variety may be distinguished from *C. malinvernianiforme* Grönbl. var. *malinvernianiforme* by its truncate to angulotruncate apices, and by the more slender proportions of the cells. The general appearance of the cells is very close to that of *C. moniliferum* except for the striate walls and the structure of the apices. Extremely fine striolations (20 per 10 μ) were seen in the Ontario specimens. Under oil immersion the striae in some specimens appeared to consist of parallel rows of punctae as shown in the insert to Fig. 21.

35. *C. moniliferum* (Bory) Ehr. var. *moniliferum* (Fig. 18) W. 29–48 μ; ap. W. 6–9 μ; L. 185–275 μ.

Manitoba: Churchill—C-156, C-181, C-262; Lake Winnipeg, Boundary Creek, Killarney (28).

Ontario: Brantford—2; Cochrane—130, 131, 153; Clute—132, 150, 151; Holland—241; Powassan—103, 124; Swastika—236, 247; Shoal Lake, Muskoka (28); Lake Nipigon (3, 4).

Quebec: (18, 19, 21, 23, 24). Maritimes: (17). Newfoundland: (52). Arctic: (11, 27, 57).

36. *C. moniliferum* var. *concavum* Klebs (Fig. 19) W. 42–46 μ; ap. W. 6–7 μ; L. 200–272 μ.

*Ontario: Cochrane—131.

Differs from the var. *moniliferum* in the absence of the median ventral swelling.

37. *C. parvulum* Naeg. var. *parvulum* (Fig. 11) W. 11–19.5 μ; ap. W. 2.5–4 μ; L. 97–147 μ.

*Manitoba: Churchill—C-177, C-181, C-188, C-189, C-190, C-204, C-207, C-223, C-246, C-256, C-266, C-293.

*Ontario: Cranbrook—3; Cochrane—130, 153; Gorrie—10; Timagami—231, 239; Holland—253; Clute—150.

Quebec: (18, 19, 24). Maritimes: (17). Newfoundland: (52). Arctic: (11, 27, 57).

38. *C. parvulum* var. *angustum* W. and G. S. West (Fig. 12) W. 7–9 μ; ap. W. *ca.* 2μ; L. 84–106 μ.

*Manitoba: Churchill—C-188.

*Ontario: Paris (16); Timagami—239.

Quebec: (18, 29).

In the original description of this variety, the Wests (54) state that it is "smaller and considerably narrower than the typical form". Later, however, they (55) include Borge's Paraguayan record (5) under the variety, although some of Borge's specimens are longer than the Wests' limits for var. *parvulum*. It must therefore be concluded that the var. *angustum* was named on the basis of its narrowness (hence its name) and not because it was "smaller". Apparently Krieger (25) has adopted this interpretation, since he expands the size limits given by the Wests and includes even narrower specimens (L./W. ratio up to 16) than did the original authors. On this basis at least some of the specimens assigned by Irénée-Marie (18) and Taylor (52) to var. *parvulum* should be transferred to var. *angustum*.

39. *C. parvulum* var. *majus* West (Fig. 10) W. 19–30 μ; ap. W. 2–4 μ; L. 171–234 μ.

*Manitoba: Churchill—C-127, C-156, C-183, C-201, C-202, C-203, C-261.

*Ontario: Clute—150, 151.

The varietal name (except for the case ending) is adopted as in Krieger (25). The variety appears to be valid but the name is doubtful since Schmidle (45) does not cite the original source. Some of the specimens in collection 150 approach *C. dianae* in the shape of the apices.

40. *C.* ***planum*** sp. nov. (Fig. 34).

Cellulis 22–24 μ lat., 183–275 μ long.; longitudine cellularum 7.5–12.5 plo latitudine; semi-cellulis attenuatis, apicibus truncatis circa 8 μ lat.; 8–15 pyrenoidibus in medio chromatophori in ordine positis; chromatophoris sex lamellis longitudinalibus; membranis cellularum laevibus. W. 22–24 μ; ap. W. *ca.* 8 μ; L. 183–275 μ (L. = 7.5–12.5 W.).

*Ontario: Michipicoten River;—MR-1.

The general outline of the cells of *C. planum* approaches that of *C. intermedium* Ralfs. It differs from the latter species in the complete absence of wall ornamentation, even when viewed under oil immersion, and also in the number of pyrenoids (8 to 15) and longitudinal ridges (six) in the chromatophore. Occasional specimens were seen with a median girdle.

41. *C. praelongum* Bréb. var. *praelongum.*

Ontario: Taft (51).

Quebec: (18, 19, 21, 24).

42. *C. praelongum* var. *brevius* W. West (stat. nov.) (Fig. 5) W. 17.5–19.5 μ; ap. W. 3 μ; L. 279–290 μ, (L. = *ca.* 15 W.).

*Manitoba: Churchill—C-223.

Krieger (25) refers to the fine striations visible with oil immersion on the walls of the var. *praelongum.* Similar striations (12–14 in 10 μ) are visible on the walls of the var. *brevius* in the Churchill collection. The striae, however, are punctate (or granulate?) as indicated in the insert to our figure. There is a suggestion that some of the "punctae" may be irregularly scattered with respect to the striae, particularly in the median region. The apices are punctate.

Krieger (25) created the variety *brevius* citing Nordstedt as the original user of the name. Nordstedt (34, 35) accords to W. West priority in the use of the epithet "*brevior*" (as fa. *brevior*). Krieger's precedent has been followed in raising the form to varietal status but West has been cited as the author.

43. (*a*) *C. pritchardianum* Arch. var. *pritchardianum* (Fig. 6) W. 26–32 μ; ap. W. 4–6 μ; L. 337–520 μ.

*Manitoba: Churchill—C-223, C-281-H.

*Ontario: Timagami—230 (plentiful); Trout Lake—234.

Quebec: (18, 19, 21, 24). Maritimes: (17). Newfoundland: (52).

In our material there are 10–12 pyrenoids per chromatophore. The interrupted structure of the striae (a series of dashes with occasional dots) is evident only under oil immersion. In some specimens the striae (12–20 in 10 μ) are continuous to the apices; in others, the apices are irregularly punctate.

43. (*b*) *C. pritchardianum* var. *pritchardianum* fa. ***laeve*** fa. nov. W. 28–32 μ; ap. W. *ca* 5 μ; L. 416–454 μ.

Striae punctaeque absunt; cetero ut in var. *pritchardianum.*

*Ontario: Timagami—230; Trout Lake—234.

44. *C. pronum* Bréb. var. *pronum* (Fig. 40) W. 9–10 μ; ap. W. *ca.* 2 μ; L. 275–329 μ.

*Ontario: Clute—132; London (16).
Quebec: (18, 21, 23, 24). Maritimes: (17).

45. *C. pusillum* Hantzch var. *pusillum* (Fig. 26) W. 9.5 μ; ap. W. *ca.* 5 μ; L. 54.5 μ.

*Ontario: Powassan—103.
This record, based on one specimen, is included with some hesitation. Whelden (57) reports *C. pusillum* var. *major* from the eastern Arctic.

46. *C. ralfsii* Bréb. var. *hybridum* Rabenh. (Fig. 53) W. 36 μ; ap. W. 6–7 μ; L. 420–540 μ, (L. = 12–15 W.).

Striae 10–12 in 10 μ, with very fine punctae between the striae; chromatophore with eight ridges and eight axial pyrenoids.
*Manitoba: Churchill—C-248.
Quebec: (18, 19, 21, 24, 29). Maritimes: (17). Arctic: (57).

47. *C. ralfsii* Bréb. var. ***kriegeri*** var. nov. (Fig. 37).

Cellulis 41 μ lat., 425–430 μ long.; apicibus (6–7 μ lat.) in conos truncatos subito contractis. Parte mediano membrani dorsuali fere recto, multo plus curvato ad apices accedens. Membrane ventrale mediano tumido. Membrane striato, striis 10 in 10 μ. Semi-cellulis attenuatis ad conos apicales accedentibus.
*Manitoba: Churchill—C-253.
This variety includes one of the plants assigned by Krieger (25; Pl. 30, Fig. 4) to *C. attenuatum* Ehr. Calculated dimensions of Krieger's plant are: L. 384 μ × W. 30 μ. According to the illustrations of Ralfs (41) and the Wests (55), *C. attenuatum* has much more abruptly contracted apices than *C. ralfsii* var. *kriegeri*. The former species has subcylindrical apices; apices of the latter variety are more conical. The contracted structure of the apices separates *C. ralfsii* var. *kriegeri* from *C. ralfsii* var. *hybridum*.

48. *C. rostratum* Ehr. var. *rostratum* (Fig. 47) W. 15–27 μ; ap. W. 3.5 μ; L. 210–389 μ.

*Manitoba: Churchill—C-190, C-202, C-203, C-256.
Ontario: Toronto (30); Muskoka (47); Lake Nipigon (2, 4).
Quebec: (18, 19, 21, 23, 24, 29). Arctic: (27, 57). Maritimes: (17.)

49. *C. setaceum* Ehr. var. *setaceum* (Fig. 48) W. 8–9 μ; ap. W. *ca.* 2 μ; L. 180-290 μ.

Ontario: Timagami—239; Mud Lake (28).
Quebec: (18, 19, 21, 23, 24, 29). Newfoundland: (52). Maritimes (17). Arctic: (27).
The presence of a girdle (as in the specimen illustrated) is unusual in this species.

50. *C. striolatum* Ehr. var. *striolatum* (Figs. 35, 36) W. 28–50 μ; ap. W. 10–12 μ; L. 215–400 μ, (L. = 7–10 W.).

Striae 4–6 in 10 μ; pyrenoids 6–10 per chromatophore; chromatophore with 6–8 ridges.

*Manitoba: Churchill—C-203 (plentiful), C-204, C-248, C-260, C-261. Ontario: Cochrane—130; Timagami—230 (plentiful), 231 (plentiful); Clute—150; Holland—253; Toronto (30).

Quebec: (6, 18. 19, 21, 24, 29). Maritimes: (17). Newfoundland: (52). Arctic.: (11, 27, 57).

The boundary between this species and *C. intermedium* appears to be somewhat vague. According to the Wests (55) and Irénée-Marie (18) the striae are more numerous in *C. striolatum* (i.e. they are closer together). Krieger (25), however, allows 6–10 striae in 10 μ for *C. intermedium* and 5–10 striae in 10 μ for *C. striolatum*.

In the author's material, the striae are quite distinct ridges, sometimes almost costae, in both species and range from 4 to 6 in 10 μ (cf. however, collection C-252 of *C. intermedium*). In both species, scattered punctae may be seen between the striae under oil immersion. In collection C-203, *C. striolatum* var. *striolatum*, C. *striolatum* var. *erectum*, and *C. intermedium* var. *hibernicum* are present. In this collection, the var. *hibernicum* is easily separated from the other two varieties by its narrowness (22–25 μ) and its greater length–width ratio (L. = 15–18 W.).

The statement in Krieger's diagnosis "Chromatophoren mit 12–13 Längsplatten" must be in error according to his Fig. 8 (25) and to the Wests (55).

In the present study, specimens with a width less than 26 μ have been assigned to *C. intermedium* and with a width greater than 28 μ to *C. striolatum*. Nearly all of the specimens of *C. striolatum* seen in collections 230, 231, and C-203 have a width of 35–40 μ.

The ventral margin of *C. striolatum* var. *striolatum* varies from concave to nearly straight. Some specimens in C-203 were almost concave enough for inclusion in Krieger's var. *borgei* but all intergradations were present. Specimens without a median girdle always appear to be more curved than those with one or more girdles.

51. *C. striolatum* var. *erectum* Klebs W. 35–37 μ; ap. W. *ca.* 10 μ; L. 375–450 μ, (L. = 10–12 W.).

*Manitoba: Churchill—C-203.

*Ontario: Holland—245; Timagami—230.

Quebec: (18, 21, 23).

The length of the cells in this variety is not always due to the addition of girdles as implied by Krieger (25). Some specimens in the Churchill and Holland collections have no girdles.

52. *C. striolatum* var. *subtruncatum* (W. and G. S. West) Krieger (Fig. 51) W. 38 μ; ap. W. 14 μ; L. 298 μ.
*Ontario: Holland—241.
Quebec: (7, 19, 24).
A comparison of the illustrations of Borge (*C. subtruncatum* fa., (5)) Schmidle (*C. regulare* var. *dilatatum*, (44)) and Sampaio (*C. limicum*, (43)) indicates that there is a great deal more variation in this variety than one would expect from the illustrations in Krieger (25), Irénée-Marie (18), and Smith (48). The degree of contraction proximal to the capitate apices is quite extreme in Sampaio's figures but barely (if at all) visible in the figures published by Iréneé-Marie and Smith. The degree of dilation of the apices also varies considerably.

53. *C. tumidum* Johns. var. *tumidum* (Fig. 43) W. 12 μ; ap. W. 4–5 μ; L. 100–102 μ.
*Northwest Territories: Ennadai Lake—J-7.
Quebec: (18, 19, 24).

54. *C. tumidum* var. *nylandicum* Grönbl. W. 9–13 μ; ap. W. 2–4.5 μ; L. 100–201 μ.
*Manitoba: Churchill—C-128, (Fig. 44); C-202, (Fig. 25).
*Northwest Territories: Ennadai Lake— J-7, (Fig. 45).
Associated with *C. tumidum* var. *tumidum*. The author is dubious about the amount of variation credited to this variety.

55. *C. turgidum* Ehr. var. *turgidum* (Fig. 59).
Ontario: Toronto (30).
Quebec: (6, 18, 19, 21, 24).
Maritimes: (17).

56. *C. venus* Kuetz. var. *venus* (Fig. 13) W. 7–14 μ; ap. W. 2–3 μ; L. 53–94 μ.
*Manitoba: Churchill—C-104, C-106, C-107, C-108, C-128, C-202, C-203, C-246, C-248, C-262.
Ontario: Cochrane—131; Gorrie—8; Holland—253; Toronto (30).
Quebec: (18, 19, 21, 23, 24, 29). Maritimes: (17). Newfoundland: (52). Arctic: (27, 57).
Some of the specimens in collection C-262 are larger than the maximum cited by Krieger (25). They were segregated from *C. parvulum* (same collection) on the basis of their greater curvature and smaller number of pyrenoids (two) per semicell.

57. *C. venus* var. *incurvum* (Bréb.) Krieger (Fig. 14) W. 8–15 μ; ap. (acute); L. 47–72 μ.
*Manitoba: Churchill—C-204.
*Ontario: Gorrie—8, 10; Brantford (collection lost).
Quebec: (18, 19, 21, 24). Maritimes: (17).

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